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PROTOCOL

"*Needle-free Jet Injection of Reduced dose, Intradermal, Influenza Vaccine in ≥6 to <24-month-old Children*"

Filename: **4785-ID-INF-Prot-Engl-2008Jun24.pdf** (prefixed with **"Cover-Page+Waiver**")

Full Study Title - English: **Clinical Trial of Safety (Reactogenicity) and Immunogenicity of Needle-free Jet Injection of Reduced-dose, Intradermal, Influenza Vaccine (INF) Administered to ≥6 -to- <24 Month-old Infants and Toddlers in the Dominican Republic**

Full Study Title - Español: **Ensayo Clínico de Seguridad y Respuesta Inmunológica de Inmunización Administrada sin Aguja con Inyector a Chorro en Dosis Reducida por vía Intradérmica con Vacuna Influenza (Gripe) (INF) en Niños de ≥6 - <24 Meses de Edad en la República Dominicana**

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PROTOCOL

Clinical Trial of Safety (Reactogenicity) and Immunogenicity of Needle-free Jet Injection of Reduced-dose, Intradermal, Influenza Vaccine (INF) Administered to ≥6 -to- <24 Month-old Infants and Toddlers in the Dominican Republic

Ensayo Clínico de Seguridad y Respuesta Inmunológica de Inmunización Administrada sin Aguja con Inyector a Chorro en Dosis Reducida por vía Intradérmica con Vacuna Influenza (Gripe) (INF) en Niños de ≥6 - <24 Meses de Edad en la República Dominicana

Centers for Disease Control and Prevention & Hospital Infantil Dr. Robert Reid Cabral

1. Title Page

Study Title:	Clinical Trial of Safety (Reactogenicity) and Immunogenicity of Needle-free Jet Injection of Reduced-dose, Intradermal Influenza Vaccine (**INF**) administered to ≥6 -to- <24 Month-old Infants and Toddlers in the Dominican Republic *[Ensayo Clínico de Seguridad y Respuesta Inmunológica de Inmunización Administrada sin Aguja con Inyector a Chorro en Dosis Reducida por vía Intradérmica con Vacuna Influenza (Gripe) (**INF**) en Niños de ≥6 - <24 Meses de Edad en la República Dominicana]*
Brief Title:	HIRRC/CDC Needle-free Intradermal Influenza Study *[HIRRC/CDC Estudio Influenza Intradérmica sin Aguja]*
Protocol Number:	CDC-ISO-4785
Investigational Product(s):	Vaxigrip® influenza vaccine (**INF**) (Sanofi Pasteur, Lyon, France), a Dominican-registered product (in prefilled syringes, but not yet in vials), to be administered "off label" by intradermal injection using the U.S.-licensed Biojector® 2000 jet injector with investigational intradermal spacer (not Dominican-registered)
Indication:	Induce protective levels of serum antibodies to influenza virus antigens after two jet-injected, intradermal, reduced volume/antigen doses of trivalent vaccine, spaced at least one month apart, in infants ≥6 to <24 months of age, as a correlate of protection from influenza disease
Sponsor:	Immunization Safety Office, Centers for Disease Control and Prevention (D-26), 1600 Clifton Road, Atlanta, GA 30333
Phase(s):	Phase I, followed by Phase II (depending on phase I safety results)
Sponsor's Responsible Medical Officer:	Bruce G. Weniger, M.D., M.P.H.

2. Signatures

INVESTIGATORS. Each of the undersigned investigators agrees to conduct the study as stated in this protocol and in compliance with all applicable obligations, regulations, and guidelines. Each agrees to inform the other investigators of all relevant information that becomes available during the conduct of this study.

[*signature*] Bruce G. Weniger — 6 March 2006
Bruce G. Weniger, M.D. U.S. Principal Investigator, Sponsor Responsible Medical Officer, and Project Director, CDC — Date

[*signature*] — 03/06/06
Virgen Gómez, M.D., Dominican Principal Investigator, HIRRC — Date

[*signature*] — 06 MAR 2006
Jesús M. Feris Iglesias, M.D. Dominican Senior Investigator, HIRRC — Date

[*signature*] — 3/7/06
Robert L. Davis, M.D., U.S. Senior Investigator, CDC — Date

[*signature*] Dra Josefina Fernandez 06-Mar-2006
Josefina Fernández, M.D., Dominican Co-Investigator, HIRRC — Date

[*signature*] — 3/7/06
Pedro Moro, M.D., U.S. Co-investigator, CDC — Date

[*signature*] — 03/06/06
Guillermo Herrera, M.D., U.S. Co-investigator, CDC — Date

[*signature*] — 3/6/06
Carolyn Bridges, M.D., U.S. Co-investigator, CDC — Date

[*signature*] — 2006-MAR-06
Martin Friede, Ph.D., Co-investigator, WHO — Date

3. Synopsis

Name of Sponsor/Company:
Immunization Safety Office, Office of Chief Science Officer, Centers for Disease Control and Prevention (E-61), 1600 Clifton Road, Atlanta, GA 30333, USA

Study number:
CDC-ISO-4785

Name of Finished Products (§10.4**):**
Vaxigrip® Inactivated Influenza Vaccine Trivalent Types A and B (Split Virion), Sanofi Pasteur, Lyon, France
Biojector® 2000 Needle-free Injection Management System [jet injector] with investigational intradermal spacer, Bioject Medical Technologies, Inc., Portland, OR, USA

Title of study:
Clinical Trial of Safety (Reactogenicity) and Immunogenicity of Needle-free Jet Injection of Reduced-dose, Intradermal Influenza Vaccine (**INF**) Administered to ≥6 -to- <24 Month-old Infants and Toddlers in the Dominican Republic *[Ensayo Clínico de Seguridad y Respuesta Inmunológica de Inmunización Administrada sin Aguja con Inyector a Chorro en Dosis Reducida por vía Intradérmica con Vacuna Influenza (Gripe) (**INF**) en Niños de ≥6 - <24 Meses de Edad en la República Dominicana]*

Investigators (§8)**:**
Bruce G. Weniger, M.D., M.P.H.; Virgen Gómez, M.D.; Pedro L. Moro, M.D., M.P.H.; Josefina Fernández, M.D.; Glenny Gúzman, M.D., C. Sarah Mota T., M.D., Jesús M. Feris Iglesias, M.D.; Carolyn Bridges, M.D.; Martin Friede, Ph.D., John K. Iskander, M.D.

Study site (§10.2)**:**
Hospital Infantil Dr. Robert Reid Cabral [national children's hospital]
Santo Domingo, Dominican Republic

Cooperating Organizations (§8)**:**
- World Health Organization, Geneva, Switzerland
- Program for Appropriate Technology in Health, Seattle, WA, USA
- Bioject Medical Technologies, Inc., Portland, OR, USA [donor of injection device]
- Sanofi Pasteur SA [donor of vaccine product]
- Laboratory facility [to be determined by WHO tender among experienced and qualified research level institutions meeting cGLP or equivalent standards]

Study period: 2006 – 2009	**Phase(s) of development:** Phase I and phase II

Study Description (§10.1)**:**

A sequential phase I and II, controlled, double-blinded study to determine whether immune responses suggesting protection against influenza can safely be induced in young children by two reduced doses one month apart of 0.1 mL of a trivalent inactivated influenza vaccine (**INF**) administered by the intradermal (ID) route with an investigational ID spacer on a U.S.-licensed needle-free jet injector (JI), compared to two standard intramuscular (IM) 0.25 mL doses by needle-syringe (N-S) in this age group. The locale is a developing country where financial restraints for the use of full-dose influenza vaccine would limit protection from influenza pandemic threat, where N-Ss pose dangers and drawbacks in clinical use, and

where Mantoux-type N-S ID injections are difficult to administer during mass campaigns.

Objectives (§9.6)**:**

The **primary endpoint** of this study is to measure the percentage of participants recruited at age ≥6 -to- <24-months with seroconversion on hemagglutination inhibition (HI) assay of serum collected at least one month after two doses of influenza vaccine (**INF**) administered in a reduced-dose volume of 0.1 mL intradermally (ID) by needle-free jet injector and intramuscularly (IM) by conventional needle-syringe (N-S), compared to standard IM injection of full 0.25 mL doses.

Secondary objectives of this study are to determine for the above comparison seroprotection rates, geometric mean titers, and the extent and frequencies of local and systemic reactions.

Methods (§10)**:**

Randomized, observer-blinded, clinical pilot (phase I) trial of safety, followed by a clinical (phase II) trial of safety and *non-inferiority* of immune response to the standard route and dose for the merged participants from both phases.

Phase I - Influenza-vaccine naïve children (n=48) aged ≥6 -to- <24 months will be randomized in a 1:1:1 ratio to the following three study arms, each to receive two doses on days 0 and 28 of trivalent inactivated influenza (**INF**) vaccine (Vaxigrip®, Sanofi Pasteur, Lyon, France) into the left thigh (<12 months) or left deltoid (≥12 months):

- Group "**ID-JI-0.1**" (n=16) - reduced 0.1 mL **INF** doses administered intradermally (ID) by needle-free jet injector (JI) (Biojector® 2000 subcutaneous syringe no. 2 (green color code), with 2cm investigational spacer, Bioject Medical Technologies, Inc., Portland, OR, USA)

- Group "**IM-NS-0.1**" (n=16) - reduced 0.1 mL **INF** doses administered intramuscularly (IM) needle-syringe (NS) (via 22-25 gauge needle, minimum 25mm/1-inch length)

- Group "**IM-NS-0.25**" (controls) (n=16) - full 0.25 mL **INF** doses administered intramuscularly (IM) by needle-syringe (NS) (22-25 gauge needle, minimum 25mm/1-inch length)

Phase II - Upon assessment of the safety profile from phase I by the unblinded Data Safety Monitoring Board (DSMB), with its approval an additional 402 children will be recruited and randomized (134 per group) as in phase I above. Total participants in phase I and II = 450 (150 in each of three study arms).

Adverse Event Diaries (§10.6.4)**:** Parents will be trained to complete a diary form to observe, measure, and record solicited local reactions for the injection site and systemic signs and symptoms for the child for days 0 through 7 after vaccination, plus unsolicited symptoms, illness, and medications for days 0 through 28.

Followup (§10.6.1)**:** Return clinic visits will be scheduled on days 2, 7, and 28 after **INF** dose 1, at which times the diary card data will be recorded by staff and the card collected on day 28. Telephone calls and/or home visits on days 3, 9, and 29 will occur for participants not returning as scheduled on the preceding day. Upon receiving dose 2 of vaccine, participants will be scheduled again to return to the study center 2, 7, and 28 days afterwards. The same procedures as for dose 1 regarding diary cards, telephone followup, and home visits will apply after dose 2. Upon returning to clinic on day 28 after dose 2 (day 56 after dose 1), the children in reduced dose groups ID-JI-0.1 and IM-NS-0.1 will receive an unblinded, "insurance", full-volume, 0.25 mL dose (#3) of influenza vaccine by NS IM. Group IM-NS-0.25 will receive a blinded mock 3rd injection. All groups will return 6 months later (8 months after dose 1, 7 after dose 2) for a "bonus" booster vaccination for the next influenza season, at which time late adverse events since the last visit will be queried.

Serum and Virus Collection (§10.6.10)**:** Blood specimens to measure serologic responses will be collected three times, just prior to vaccination on day 0 (**INF** dose 1), on day 28 (**INF** dose 2), and on day 56 (**INF** "insurance" dose 3). Participants with influenza-like illness will have nasopharyngeal/throat swabs analyzed by rapid influenza test. Those with positive influenza A results will have specimens sent for viral culture.

Stopping rules (§10.9)**:** Vaccination will be suspended for all participants, pending unblinded review by the DSMB and its clearance or denial to resume, as well as simultaneous IRB guidance, upon occurrences deemed *possibly-* or *probably-related* to the study vaccinations by any of the Dominican or U.S. Principal or Senior Investigators of *serious adverse events* in any 3 (6%) phase I vaccinees or any 16 (4%) phase II vaccinees, or upon incidents of *entire-limb swelling* or other *severe local reactions* at the **INF** injection site in any 5 (10%) phase I vaccinees or any 20 (5%) phase II vaccinees.

Criteria for proceeding to phase II (§10.10)**:** Either or both of the study's investigational arms (**ID-JI-0.1** or **IM-NS-0.1**) will not proceed to phase II if the observed frequency of the following events in that arm exceeds the frequency observed in the control **IM-NS-0.25** group by the designated excess:

	(Invest. Arm) – (Control Arm)
• Any *serious adverse event*, *possibly- or probably-related*:	exceeds by ≥3
• Fever >40º C *possibly- or probably-related*:	exceeds by ≥4
• Injection site whole-limb swelling:	exceeds by ≥4
• Injection site induration >5 cm diameter:	exceeds by ≥5
• Injection site erythema >5 cm diameter:	exceeds by ≥5
• Injection site pain level 3 (cries upon moving limb):	exceeds by ≥5

Number of participants planned:

Phase I = 48; phase II = 402 (total participants = 450)

Participant characteristics and main criteria for inclusion and exclusion (§10.3)**:**

Healthy, full-term infants of age ≥6 -to- <24 months (before second birthday) who are recruited as prior or current patients, or siblings of patients, attending a public outpatient clinic at the national children's hospital. Excluded will be infants not up-to-date on immunizations who need other injected vaccines recommended routinely at younger ages, or

those with chronic or acute clinical illness contraindicating vaccination, or residence outside the metropolitan area (*Distrito Nacional*) whence return visits would be inconvenient.

Investigational vaccine(s), antigen content, dosage, route of administration (§10.4.2)**:**

Inactivated Influenza Vaccine Trivalent Types A and B (Split Virion) (**INF**) (Vaxigrip®[a], Sanofi-Pasteur, Lyon, France), containing 15 µg hemagglutin (HA) per 0.5 mL for each of three virus strains (2006 Southern Hemisphere formulation):

- A/New Caledonia/20/99 (H1N1) [IVR-116]
- A/New York/55/2004 (H3N2) [NYMC X-157] (an A/California/7/2004-like strain)
- B/Victoria/2/87 lineage (a B/Malaysia/2506/2004-like strain)

When the initial season formulation expires, new participants will be vaccinated with either the 2006-2007 Northern Hemisphere and/or the succeeding 2007 Southern Hemisphere formulations. If these formulations differ in their virus strains, recruitment will be deferred to ensure each participant receives unexpired doses from the same formulation (serologic assays will be segregated accordingly).

The investigational regimen will be a reduced dose of 0.1 mL (3 µg HA per virus strain) injected into the left thigh (or left deltoid for age ≥12 months) by either routine intramuscular (IM) needle-syringe (N-S) injection, or by needle-free Biojector® 2000 jet injector (JI) with investigational spacer for intradermal (ID) delivery (Bioject Medical Technologies, Portland, OR, USA)[b]. Same dose repeated one month later. One month after that, all participants receive unblinded third "insurance" dose of full 0.25 mL by N-S IM.

Reference vaccine, antigen content, dosage regimen, route of administration:

For the control group, the same **INF** vaccine as above will be administered in standard 0.25 mL dosage (7.5 µg HA per virus strain) for this age group into the left thigh (or left deltoid for age ≥12 months) IM by conventional N-S. Same dose repeated one month later. One month after that, all participants receive unblinded third "insurance" dose of full 0.25 mL by N-S IM.

Concomitant vaccines (§10.4.7)**:**

No other routine or investigational vaccines will be administered within 28 days before or after either of the study's investigational **INF** doses 1 and 2 described above. Participants who may have received, or are due to receive recommended vaccines such as **POL_{OPV}** and **DTP_w-HBV-HIB** (recommended at 6 and 18 months) and **MMR** (12 months) will not be recruited during appropriate intervals before and after these routine doses.

Measurement of Immunogenicity (§10.5.1)**:**

(See primary and secondary endpoints in **Objectives** section, above.)

Tenders for performance of serologic assays will be solicited by WHO from the Laboratory for Specialized Clinical Studies, Division of Infectious Diseases, Cincinnati Children's

[a] As of 8 June 2006, Sanofi Pasteur's Vaxigrip® influenza vaccine was licensed by the regulatory authority of the Dominican Republic, the Dirección General de Drogas y Farmacias, for routine sale and use only in its prefilled syringe packaging format, but not in its 5 mL multidose vials. Vaxigrip® is licensed in Canada and many other countries of Latin America, the Caribbean, Europe, Africa, and Asia, but not in the United States.

[b] As of 8 June 2006, Bioject's Biojector® 2000 jet injector was licensed as a medical device in the U.S., Canada, and other countries of the Americas, Europe, and Asia, but not in the Dominican Republic.

Hospital Medical Center, Cincinnati, OH, USA; from the National Institute for Biological Standards and Controls, Potters Bar, Hertfordshire, UK; and from other qualified research laboratories via public solicitation.

Hemagglutination inhibition (HI) assays against viral strains similar to those included in the vaccine will be performed according to current Good Laboratory Practices (cGLP) and standard assay protocol. Assay antigens will be selected for suitability with strains in the vaccine formulations. After treatment to eliminate nonspecific inhibitors, specimens will be diluted 2-fold through a final dilution of 1:1024 or higher. Additional serum controls diluted from each specimen and red blood cell controls will be used on each plate. Viral strain antigens -- obtained from external reference laboratories or prepared on site -- will be prepared daily when assays are performed.

Measurements of safety (§10.5.2)**:**

- Investigator-observed local injection site and systemic symptoms at 0 and 30-60 minutes, plus 2, 7, and 28 days after both the injections. Home telephone calls and home visits, if needed, by study staff on days 3, 9, and 29 for participants failing to return to clinic as scheduled.
- Prompted daily measurement and recording on parental diary form for 8 days following both study injections of local (tenderness, erythema, induration, ecchymosis, circumference) and systemic reactions (fever, anti-fever medication, loss of appetite, sleepiness, irritability, unusual or inconsolable crying, vomiting, diarrhea, convulsions, other to be specified).
- Unprompted reports on parental diary form or by telephone of these or other adverse events or symptoms occurring up to 28 days after each injection, plus report upon return for "bonus" booster vaccination 6 months after dose 2 + 28 days.

Statistical power considerations (§10.8)**:**

For each of three treatment arms in the combined phases I and II, 150 participants will be studied, resulting in a total sample size of 450. This number results from rounding up, in anticipation of modest loss-to-followup, from a calculated 142 participants required per arm.

The sample size calculation is based on a *non-inferiority* model using an expected seroconversion rate of 86.4% derived from a mean from four prior clinical studies of Vaxigrip® in the similar age of range of 6 to 36 months. A twelve percent decrement, i.e., 0.104, is selected, corresponding to an *acceptable* seroconversion rate as low as 76.0% in the investigational study arms to demonstrate they are not worse than the control arm by more than this amount. Sample size parameters are $\alpha = 0.05$, *Power* = 0.80 ($\beta = 0.2$).

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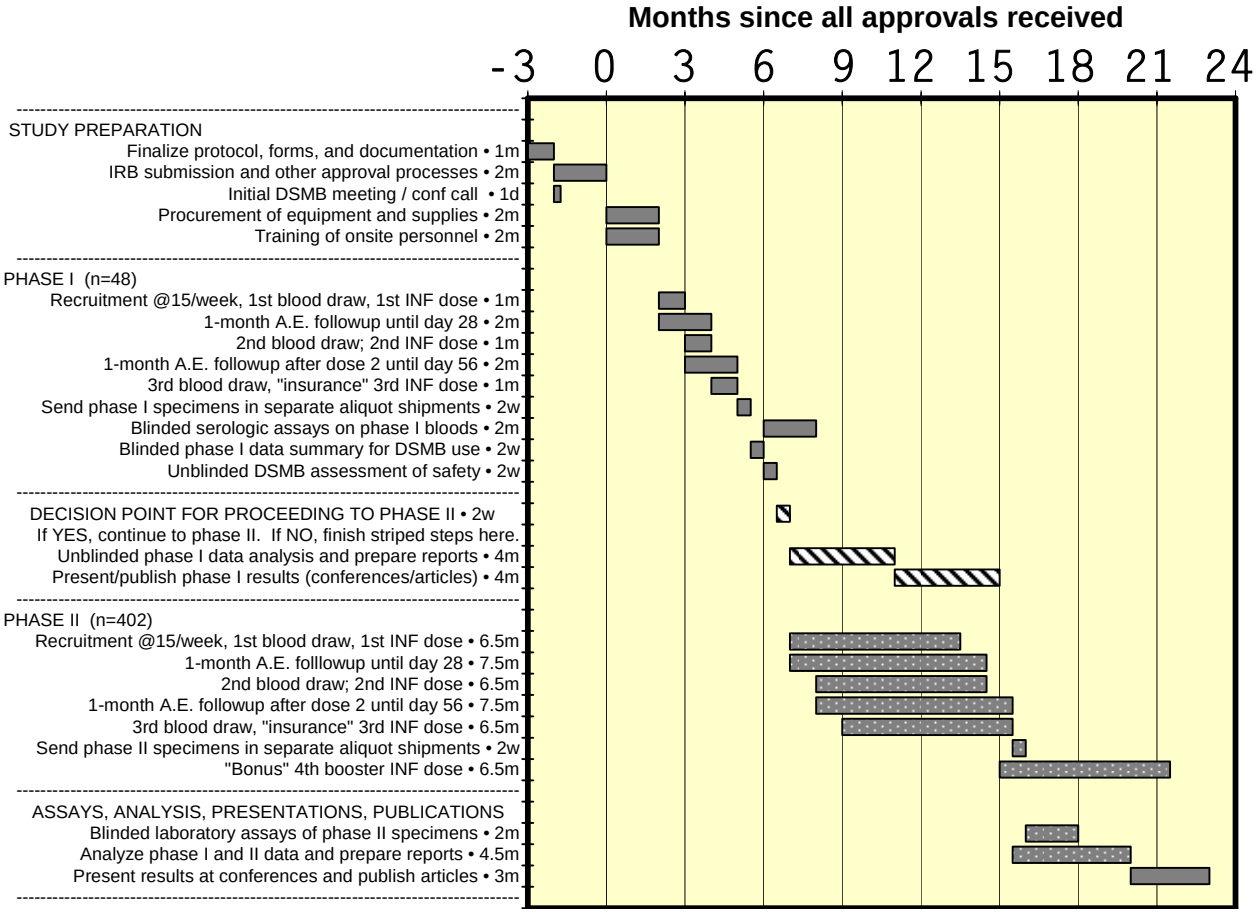
5. Study and Events Timeline

Figure 1. Study and events timeline.


Months since all approvals received
-3 0 3 6 9 12 15 18 21 24
STUDY PREPARATION
Finalize protocol, forms, and documentation • 1m
IRB submission and other approval processes • 2m
Initial DSMB meeting / conf call • 1d
Procurement of equipment and supplies • 2m
Training of onsite personnel • 2m
PHASE I (n=48)
Recruitment @15/week, 1st blood draw, 1st INF dose • 1m
1-month A.E. followup until day 28 • 2m
2nd blood draw; 2nd INF dose • 1m
1-month A.E. followup after dose 2 until day 56 • 2m
3rd blood draw, "insurance" 3rd INF dose • 1m
Send phase I specimens in separate aliquot shipments • 2w
Blinded serologic assays on phase I bloods • 2m
Blinded phase I data summary for DSMB use • 2w
Unblinded DSMB assessment of safety • 2w
DECISION POINT FOR PROCEEDING TO PHASE II • 2w
If YES, continue to phase II. If NO, finish striped steps here.
Unblinded phase I data analysis and prepare reports • 4m
Present/publish phase I results (conferences/articles) • 4m
PHASE II (n=402)
Recruitment @15/week, 1st blood draw, 1st INF dose • 6.5m
1-month A.E. folllowup until day 28 • 7.5m
2nd blood draw; 2nd INF dose • 6.5m
1-month A.E. followup after dose 2 until day 56 • 7.5m
3rd blood draw, "insurance" 3rd INF dose • 6.5m
Send phase II specimens in separate aliquot shipments • 2w
"Bonus" 4th booster INF dose • 6.5m
ASSAYS, ANALYSIS, PRESENTATIONS, PUBLICATIONS
Blinded laboratory assays of phase II specimens • 2m
Analyze phase I and II data and prepare reports • 4.5m
Present results at conferences and publish articles • 3m

6. List of Abbreviations and Definition of Terms[c]

AE	Adverse event
CDC	Centers for Disease Control and Prevention
CPMP	Committee for Proprietary Medicinal Products, of the EMEA
CONABIOS	Consejo Nacional de Bioética en Salud [National Council on Bioethics in Health, an organ of SESPAS]
CRF	Case report form
DCF	Data clarification form
DSMB	Data Safety Monitoring Board
DTP_W	Diphtheria, tetanus, pertussis (whole cell) combination vaccine
EC	ethics committee (generic term for institutional review board; see IRB)
EIA	Enzyme immunoassay
EMEA	European Medicines Agency (formerly know as the European Agency for the Evaluation of Medicinal Products)
FDA	Food and Drug Administration
FDI	*Fundación Dominicana de Infectología* [Dominican Infectious Disease Foundation]
GAVI	Global Alliance for Vaccines and Immunizations
GSK	GlaxoSmithKline, vaccine manufacturer
GCP	Good Clinical Practice; also cGMP: current Good Clinical Practice
GLP	Good Laboratory Practice; also cGLP: current Good Laboratory Practice
GMT	Geometric mean titer
HIRRC	Hospital Infantil [Children's Hospital] Dr. Robert Reid Cabral
HI	Hemagglutination inhibition [assay]
ICH	International Conference on Harmonization
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional review board
ISO	Immunization Safety Office, unit of OCSO
JI	Jet injector, jet injection, or jet-injected
JI-IM	jet-injected intramuscular administration
JI-SC	jet-injected subcutaneous administration
MEA	Measles virus vaccine
MEN	Meningococcal (*Neisseria meningitidis*) vaccine, not otherwise specified
$MEN_{ps\text{-}AC}$	Meningococcal (*Neisseria meningitidis*) vaccine, polysaccharide, serogroups A and C
MMR	Measles, mumps, rubella virus combination vaccine
NIP	National Immunization Program, unit of CDC
N-S	Needle-syringe
NS-IM	needle-syringe intramuscular administration
OCSO	Office of the Chief Science Officer, CDC
OPS	Organización Panamericana de la Salud [PAHO]
PAHO	Pan American Health Organization [OPS]
PI	Principal Investigator (U.S. or Dominican)
POL_{OPV}	Poliomyelitis vaccine, live attenuated oral "Sabin" type
Sn-Pa	Sanofi-Pasteur, vaccine manufacturer subsidiary of sanofi-aventis Group

[c] Vaccine abbreviations in this table and elsewhere in the document conform to the recommendations of the Vaccine Identification Standards Initiative (http://www.cdc.gov/nip/visi/prototypes/vaxabbrev.htm)

SAR	**MEA,** measles (*sarampión* in Spanish) virus vaccine
SRP	Abbreviation in Spanish for *sarampión* (measles), *rubéola* (rubella), *paperas* (mumps) combination virus vaccine, which in English is **MMR (**measles, mumps, rubella)
SC	Subcutaneous, or seroconversion, depending on context
SESPAS	Secretaría de Estado de Salud Pública y Asistencia Social [ministry of health equivalent]
SI	Senior Investigator (U.S. or Dominican)
SP	Seroprotection

7. Ethics

7.1 Institutional Review Boards or Independent Ethics Committees

The principal investigators will provide the institutional review board (IRB)/ethics committee (EC) of both the Fundación Dominicana de Infectología (FDI), serving the Hospital Infantil Dr. Robert Reid Cabral (HIRRC) [national children's hospital], and of the Centers for Disease Control and Prevention (CDC) with all appropriate material, including the informed consent document.

The trial will not be initiated until appropriate approvals of the protocol, the informed consent document, and all recruiting materials are obtained in writing from the IRB/ECs of both the FDI and the CDC. In addition, before implementation, the study will be submitted for requisite approval by the Consejo Nacional de Bioética en Salud (CONABIOS), the ethical review body of the Secretaría del Estado de Salud Pública y Asistencia Social (SESPAS) [ministry of health equivalent]. In anticipation of supplementary funding for the study, the Ethics Review Committee of the World Health Organization (WHO), Geneva, Switzerland, will also review the documentation (logged as RPC170).

Appropriate reports on the progress of the study will be made to both the FDI and CDC IRBs (and to authorized others so requesting) by the principal investigators in accordance with applicable governmental regulations and in agreement with policy established by the sponsor.

The IRB #1 of the Fundación Dominicana de Infectología, Inc. (parent organization IORG0004207) was registered as no. IRB00004986 with the U.S. Office for Human Research Protections on 8 August 2005 (renewal scheduled 8 August 2008), under a Federalwide Assurance no. FWA00008924 approved on 11 August 2005 (expiration 11 August 2008).

IRB nos. 1-A, 2-B, 3-C, 6-G, and 7-S of the Centers for Disease Control and Prevention (CDC) (parent organization IORG 0000112) were re-registered as IRB nos. IRB00000183, -0184, -0185, -0188, and -2724, respectively, on 27 February 2004 (expiration 27 February 2007), under FWA no. FWA00001413, approved on 6 November 2001 (expiration 5 April 2008).

7.2 Ethical Conduct of the Study

This study will be conducted in accordance with the Declaration of Helsinki and good clinical practice (GCP) guidelines of the International Conference on Harmonization. Specifically, this study is based on adequately performed laboratory and animal experimentation (by others); will be conducted under a protocol reviewed by an IRB or EC; will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to its risks; the rights and welfare of the participants will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each participant's parent or legal guardian will give his or her informed consent.

7.3 Participant Information and Consent

A properly executed, written, informed consent, in compliance with GCP according to ICH guidelines, will be obtained from the parent or legal guardian of each participant minor prior to entering the participant into the trial. The consent form was pre-tested for understanding among an informal sample of parents bringing children for care at the HIRRC, and was revised accordingly before finalization. A copy of the informed consent document will be submitted by the investigator to the IRB/ECs for review and approval prior to the start of the study. The investigators will provide a copy of the signed informed consent form to each participant's parent or guardian, and will maintain a copy in the participant's record file. A social worker will observe the informed consent process, talk privately with a voluntary sample of parents, and advise the investigators of any needed improvement in the process (see section 10.6.4).

The informed consent document will explain and provide for a separate written signature for permission to maintain indefinite storage of leftover blood or swab specimens not needed in the study assays for future testing (except for HIV and human genetics). Parents may decline such signature without affecting their participation in the study.

7.4 Obligation to Participants for Related Adverse Consequences

Any participants suffering medical harm as a consequence of participation in the study shall receive from the HIRRC regular or intensive medical care for such harm, as needed and as normally available from the HIRRC for its other patients, without limitation as to the duration of such care, and without charge for such care to the participant or to his/her family (see section 11).

7.5 Insurance

Prior to the start of participant enrollment and vaccination, an insurance policy will be purchased by the World Health Organization and put into effect on behalf of the collaborating institutions conducting this study (HIRRC, FDI, CDC, and WHO) to cover liability for harm to participants which may result as a direct consequence of participating in this study (see details in section 11.1).

7.6 Level of Risk for Research on Children

In accordance with the Code of Federal Regulations (45 CFR 46.405), the investigators suggest that this research should be classified as *"greater than minimal risk"* because all vaccination carries some risk for usually mild and self-limiting local (e.g., swelling, soreness) and systemic (e.g., fever, irritability) reactions. However, there is *"the prospect of direct benefit to the individual subjects"* because of the likely protection that will be conferred on participants from the known morbidity and mortality of influenza disease as a result of trial participation.

Thus, under 46 CFR 46 Subpart D, this minimal risk is justified by the anticipated benefit to participants, and such anticipated benefit is at least as favorable as any alternative approaches to preventing influenza, of which there are none in these circumstances. Although it is not possible to solicit the assent of children of the target ages, the informed permission of their parents/guardians will be a requirement of enrollment.

7.7 Regulatory Oversight of Investigational Products

The study will not proceed until written authorization for use in the trial of the Vaxigrip Vaccine in its vial packaging and "off-label" route and dose, and of the Biojector® 2000 jet injector with investigational intradermal spacer, is received from the national pharmaceutical regulatory authority of the Dominican Republic, its General Directorate of Drugs and Pharmacies (*Dirección General de Drogas y Farmacias*), a unit of its ministry of health, the *Secretaría de Estado de Salud Pública y Asistencia Social* (Secretariat of State of Public Health and Social Assistance, abbreviated SESPAS). FDA regulatory oversight is precluded (see section 12.4).

8. Investigators and Study Administrative Structure

The trial will be overseen by staff of the Immunization Safety Office (ISO), Office of the Chief Science Officer, U.S. Centers for Disease Control and Prevention (CDC), and the National Center for Immunization and Respiratory Diseases (NCIRD), CDC. It will be jointly planned and clinically administered by employees or representatives of the Fundación Dominicana de Infectología (FDI) and the affiliated Hospital Infantil Dr. Robert Reid Cabral (HIRRC) [national children's hospital] of the Dominican Republic. Technical and financial input is contributed by the World Health Organization and the Program for Appropriate Technology in Health.

Employees or representatives of the manufacturer of the jet injector and investigational spacer to be used, Bioject, Inc. and the vaccine manufacturer, sanofi pasteur SA, will assist with provision of supplies, equipment, and/or related training and consultation. In collaboration with HIRRC, staff of CDC will perform verification of source documentation for each participant. HIRRC and CDC will be responsible for the timely reporting of serious adverse events (SAEs) to the necessary authorities and the corresponding manufacturers.

8.1 U.S. Investigators

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8.2 Dominican Investigators

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[e] WHO's participation includes (1) scientific and technical input in study design, (2) travel funding to assist CDC staff in international planning and oversight, (3) purchase on the study's behalf of liability insurance for the participating institutions (section 7.5), and (4) contracting upon competitive tender for laboratory services to perform the serologic assays and virus identification needed for the study (section 10.6.12.1).

[f] PATH is providing technical consultation and funding up to $30,000 to the Dominican collaborators for equipment and supplies needed for the study.

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9. Introduction and Background

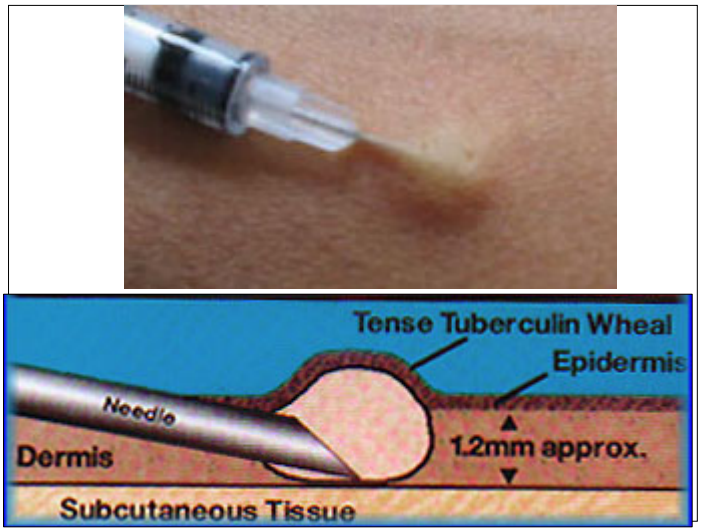
9.1 Pandemic Preparedness – Utility of Intradermal Jet Injection

Were a pandemic of high-mortality influenza to occur, developing countries will be challenged in protecting their most vulnerable populations with the limited amounts of vaccine that would likely be available or affordable. Administering reduced doses of

various antigens via the intradermal (ID) route have often found immune responses to be equivalent to full doses administered into conventional intramuscular (IM) or subcutaneous (SC) target tissues (e.g., for rabies,[Briggs2000, Wilde2005] hepatitis B,[Wahl1987, Bryan1992] and influenza,[Halperin1979] among other vaccines). One hypothesis explains this phenomenon by the skin's rich endowment with antigen-presenting dendritic (Langerhans) cells, which upon activation migrate to deeper lymphoid tissues for the next steps in the immune response.[Goldsby2003]

Such dose-sparing with ID-administered influenza vaccine (**INF**) may be a useful strategy to protect greater proportions of susceptible populations with scarce antigen.[Avison1973] For example, reduction of the standard dose of **INF** from 0.25 mL (for young children) and from 0.5 mL (for adults) to the usual intradermal volume of 0.1 mL can extend the protection of vaccine to 2½ and 5 times as many people, respectively, who would otherwise remain vulnerable to influenza morbidity and mortality.

Figure 2. Intradermal (ID) injection by Mantoux method with needle-syringe



INF vaccination in the face of a pandemic threat will likely require mass campaigns in which a limited number of trained health workers would need rapidly to vaccinate large populations in limited periods of time. ID vaccination with needle-syringe (N-S) by the traditional Mantoux method (see Figure 2), as used for PPD tuberculin application, would severely constrain mass campaigns because of the difficulty and tediousness of this technique, which requires practiced health workers and time. Needle-free jet injectors, however, have a history of rapidly and easily administering tens of millions of doses of ID vaccines, primarily for smallpox (see Figure 3), but also BCG, using specialized intradermal nozzles. Use of jet injectors for vaccination reduces the dangers and drawbacks of needle-syringe injection (see section 9.2), including intentional or inadvertent unsterile reuse, needle-stick injuries to health workers, and the unsafe disposal of sharps waste.

Figure 3. Intradermal smallpox vaccination by jet injection, simultaneous with subcutaneous measles vaccination

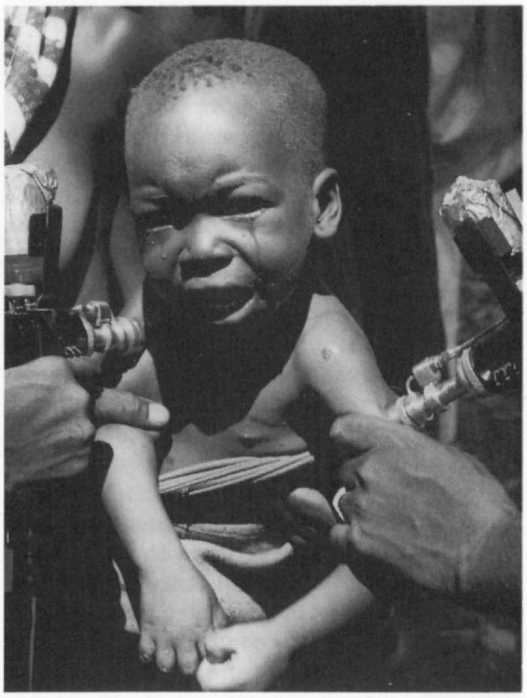


Plate 17.5. Jet injectors were used to administer smallpox vaccine in the left arm and measles vaccine in the right arm. Sometimes the injections were given simultaneously. Aluminium foil covers the vaccine vials to prevent the inactivation of virus by exposure to light.

9.2 Dangers of Unsafe Needle-syringe Injections

Only recently has the magnitude of unsafe injection practices in developing countries been widely recognized.[Holding1998] The World Health Organization (WHO) estimates that up to half of all injections in the world are unsafe because the needle or syringe has been improperly reused without sterilization.[Simonsen1999, Kane1999]

Transmission of blood borne pathogens such as human immunodeficiency virus (HIV) and hepatitis B virus to patients, healthcare workers and community members can occur from unsterile injections, accidental needle sticks, and improper "recycling" of needles and syringes.[Aylward1995a] A study which modeled unsafe injections found that one nonsterile reuse of each clean needle and syringe would result in 980 new cases of hepatitis B for every 100,000 fully immunized infants in areas of high hepatitis B prevalence.[Aylward1995b] This rate increased to 3740 cases of hepatitis B per 100,000 if each sterilized or new needle was reused just four times.[Simonsen1999] For HIV, an unsterile needle reused four times in areas where the HIV prevalence is 20% was estimated to cause up to 190 new cases of HIV infection per 100,000 fully immunized patients.[Aylward1995b]

Causes of improper use of needles and syringes include (1) inadequate training, knowledge, and motivation of health staff, (2) frequent shortages of supplies or fuel and the disrepair of equipment to sterilize needles and syringes, (3) inadequate disposal policies and facilities, and (4) a black market of recycled syringes used in the informal medical sector. To address the problem WHO and the United Nations Children's Fund (UNICEF) recommend the use of auto-disabling disposable syringes that cannot be reused.[WHO1997] But the additional cost, compared to conventional syringes, has been a barrier to their use.

The Global Alliance for Vaccines and Immunization (GAVI), created with the initiative and financial support of the Gates Foundation and others, has focused attention on vaccination technology. Among its priorities are knowledge, methods, and products to reduce the dangers of re-use of injection equipment and to ensure proper management of "sharps" waste.[Birmingham2000, Jacobs2001]

9.3 Needle-free Jet Injection Technology

9.3.1 Description and Clinical History

Needle-free jet injection offers one potential solution to the dangers and drawbacks of using needles and syringes to administer vaccines. Jet injectors (once referred to as "jet guns") use high pressure to deliver a fine stream of liquid medication or vaccines through the skin. Such devices have been used by patients themselves, by immunization clinics, and in mass vaccination campaigns since the late 1940's and early 1950s successfully to administer a wide variety of medications (e.g., anesthetics, antibiotics, corticosteroids, hormones, immunodiagnostics, immunomodulators, and vitamins) as well as vaccines.[Reis1998; NIP2005a] At least 15 million doses of measles vaccine were administered by jet injectors equipped with subcutaneous nozzles from 1967-1969 alone (along with 92 million smallpox vaccine doses via intradermal nozzles in the opposite arm) in West Africa's smallpox eradication program (Figure 3).[Millar1969, Fenner1988] In Brazil, tens of millions of doses of smallpox vaccine in the 1960s,[Veronesi1966] and tens of millions more measles vaccine doses in the 1990s[deQuadros1998] were given by jet injectors in Brazil's successful campaigns against those diseases.

The fluid injected by jet injectors is generally distributed conically, following paths of least resistance into either the subcutaneous (SC) tissues, or further into intramuscular (IM) tissue. The site of deposition where most of the dose is delivered – SC, IM, or intradermal (ID) – depends on such variables as the power of the device, its orifice size, shape, distance and angle relative to the skin, the viscosity of the fluid, the angle of injection relative to the muscle fascia plane, the skin thickness injected, and other factors.[Bennett1971, Schramm2002] Even while using the same power source, orifice, and perpendicular injection as for IM or SC delivery, ID injection can be achieved by creating a gap with tubing or a spacer inserted between the nozzle orifice and the skin, thus reducing the force of the jet stream (see section 9.6.3).[Meyer1964, Kalabus1967, Dull1968, Zsigmond1999a, Zsigmond1999b, Sugibayashi2000, Med-E-Jet2006]

9.3.2 MUNJIs vs. DCJIs

Safety concerns arose over **multi-use-nozzle jet injectors** (MUNJIs), example illustrated in Figure 3, which use the same nozzle to inject consecutive patients without intervening sterilization. A hepatitis B outbreak in the mid 1980s caused by one MUNJI,[CDC1986, WHO1986, Canter1990] as well as other published[Kremer1970, Darlow1970, Brink1985, Zachoval1988, Lukin1997, Weintraub1998, Hoffman2001, Souto2001] and unpublished[deSouzaBrito1994] studies of this and other devices, indicated blood and tissue fluid containing pathogenic agents could be transmitted among patients. This led to discontinuation and recommendations against their use in public health,[CDC2002, WHO1997] and market removal in 1997 of the most common device, the Ped-O-Jet®.[NIP2005a]

Since the 1990s, a new generation of safer **disposable-cartridge jet injectors** (DCJIs) have appeared. DCJIs avoid the inherently unsafe design of MUNJIs, since the disposable cartridge has its own sterile orifice and nozzle and is discarded between patients. One such DCJI device is the Biojector® 2000 (http://www.bioject.com/biojector2000.html), which has been studied in a number of clinical trials (see section 10.4.3), and is licensed in the U.S., Europe, and elsewhere for either subcutaneous or intramuscular injection, depending on cartridge orifice size. (A disposable investigational spacer applied to the nozzle of its disposable cartridge is to be studied by this protocol, and is described in detail in section 10.4.3.4).

9.3.3 Jet Injectors in Children

Needle-free injections have been studied and used before in pediatric populations. The Biojector® 2000 is used routinely for immunization of infants, toddlers, and older children in a number of county health department clinics in the U.S. (http://www.bioject.com/biojector2000.html). For example, the public health clinics of Cobb County, a suburban jurisdiction just outside of Atlanta, Georgia, has been using jet injectors for several years for all routine childhood immunizations, including vaccines for diphtheria-tetanus-pertussis (**DTP)**, *Haemophilus influenzae* type B (**HIB**), and hepatitis B (**HBV**) (personal communications: Richard Stout, Bioject, Inc., 1999 and Jan Smith, Cobb County Immunization Program, 1999).[Dodson1997]

As a result of the abandonment of MUNJIs in the late 1990s as described in section 9.3 above, since 2000 the U.S. Navy and Coast Guard have used the Biojector® 2000 to administer vaccines to both military recruits at basic training sites, as well as to pediatric and adult dependents at regional health facilities. In the year from October 2003 to October 2004, nearly half a million Biojector cartridges were thus used by the military (personal communication, Kurt Lynam, Bioject, Inc., 2004).

Another DCJI device, the INJEX® 50 (http://www.injex.com/products/injex30.asp), has been studied in the administration of **MMR** vaccine to teenagers and found to produce satisfactory immune responses and no significant difference in pain score compared to

control needle injections.[Sarno2000] The INJEX®, however, is not used for routine vaccination in the United States because it currently lacks capability for intramuscular injection, which are recommended for several common vaccines.

9.3.4 Immunogenicity of Jet-injected Vaccines

A large body of clinical literature documents jet injector immunogenicity, which is usually equal to or better than that induced by conventional needle and syringe for a variety of conventional inactivated and live vaccines.[Reis1998; NIP2005b] Vaccines that have been successfully administered via jet injection include typhoid,[ParentduChatelet1997] cholera,[McBean1972, PhillipinesCholeraCommittee1973] bacille Calmette-Guérin (**BCG**),[Parker1948] typhoid-diphtheria (**Td**),[Wegmann1976] whole cell diphtheria-tetanus-pertussis (**DTP**),[Stanfield1972, Ruben1973, ParentduChatelet1997, Rossier1998] measles,[Meyer1964, Ruben1973] meningococcal A and C,[Rey1989] smallpox,[Meyer1964, Veronesi1966, Ruben1973, Fenner1988] yellow fever,[Meyer1964, Ruben1973] hepatitis A,[ParentduChatelet1997, Fisch1996] hepatitis B,[Lemon1983, Whittle1987, Mathei1997] influenza,[Davies1969, Spiegel1994a, ParentduChatelet1997] plague,[Lipson1958] polio,[Lipson1958] and tetanus.[Veronesi1966, Rey1967, Rey1973, ParentduChatelet1997, Schlumberger1999]

The reported increased immunogenicity via jet injector may result because injection inevitably leaves a small amount of vaccine in the skin, which is richly endowed with dendritic (Langerhans) cells which have important roles in processing and presenting antigens in the immune system.[ParentduChatelet1997] Various studies have suggested this improved immunogenicity may allow lower doses of vaccine to be administered. For example, Hendrickse, et al demonstrated adequate levels of protective antibodies against measles after administration SC of a reduced dosage with the most widespread device, the Ped-O-Jet®.[Hendrickse1968, Macintosh1977]

9.3.4.1 Non-adjuvanted Vaccines Delivered by Jet Injection

9.3.4.1.1 Influenza Vaccines

Since the adaptation of needle-free jet injectors for mass vaccination in the early 1950s, there has been a long and well-documented history for comparable immunogenicity (to needle-syringes) and tolerable reactogenicity in their use for the administration of influenza vaccines.[Anderson1958, Clark1965, Wright1968, Davies1969, Vibes1971, Ivannikov1980, Spiegel1994a, ParentduChatelet1997] During the swine influenza mass campaign of 1976-1977 in the U.S., a substantial proportion of the approximately 80 million doses administered that season were delivered by jet injectors (CDC, unpublished data).[Ehrengut1977]

9.3.4.1.2 Measles Vaccines

Studies have well documented the immune response of the hundreds of millions of doses of measles vaccine administered by jet injectors, particularly in mass campaigns in Africa and South America (see section 9.3.1).[Meyer1964, Kalabus1967, Ruben1973, Kok1983, deQuadros1998]

9.3.4.1.3 Meningococcal Vaccines

Traditional meningococcal polysaccharide (**MEN_{ps}**) vaccines have been extensively administered by needle-free jet injection technology.[AmatoNeto1974, Artenstein1971, Binkin1982; Chippaux1998, Gotschlich1972a, Gotschlich1972b, Greenwood1980, Mohammed1981, Mohammed1984, Rey1989, Spiegel1994b, Taunay1974, Taunay1978] Most of these citations reported on the use of monovalent serogroup A polysaccharide vaccine, which more commonly causes serious epidemics in the so-called "meningitis belt" of western Sub-Saharan Africa. Amato Neto *et al*, both Taunay *et al* reports. and Rey *et al*. describe the administration of meningococcal C vaccine by this method.

9.3.5 Reactogenicity of Jet-injected IM and SC Vaccines

The medical literature reports varying results in studies regarding the pain and reactogenicity of needle-free injectors compared to needles to deliver intramuscular (IM) and subcutaneous (SC) injections. Insulin and other non-irritating drugs and non-adjuvanted vaccines generally result in either reduced or equivalent pain for jet injectors compared to needles,[Hingson1947, Hughes1949, Anderson1958, Kutscher1962, Meyer1964, Greenberg1995] but not always.[Jackson2001]

Vaccines with irritating adjuvants like aluminum salts usually result in somewhat higher frequencies of local reactions (e.g., edema, erythema, tenderness) when jet injected, but this has not generally been of a magnitude sufficient to compromise clinical tolerance and safety.[Barrett1962, Lenz1966, Edwards1974, Agafonov1974, Agafonov1978, Hoke1992, Hoke1995, ParentduChatelet1997] The irritation probably results from the residual vaccine remaining in the skin and superficial subcutaneous tissues, even if most of the dose administered is deposited more deeply. (Most modern influenza vaccines, including Vaxigrip®, have no adjuvant.)

9.3.5.1 Alum-adjuvanted Vaccines Delivered by Jet Injection

In addition to some of the papers mentioned in preceding sections, a number of reports have been published on the administration by jet injection of the following alum-adjuvanted vaccines by either the IM or SC routes. In general, these papers reported somewhat increased -- but tolerable -- rates of local reactions compared to needle-syringe administration. Such reactions are usually mild and resolve within days without treatment.

- Diphtheria-Tetanus-Pertussis (whole-cell) (**DTP_{w}**)[Stanfield1972, Ruben1973, ParentduChâtelet1997]
- Hepatitis A (**HAV**)[Hoke1992, Hoke1995, Parent du Châtelet1997, Williams2000]
- Hepatitis B (**HBV**)[Lemon1983, Whittle1987, Mathei1997]
- Tetanus (**TET**)[Veronesi1966, Rey1967, Rey1973, Parent du Châtelet1997, Schlumberger1999]
- Tetanus-Diphtheria (**Td**)[Wegman1976]
- Tetanus-Diphtheria-polio (**$Td\text{-}POL_{IPV}$**)[Barrett1962]

- Typhoid (**TYD**)[Agafonov1974, Edwards1974, Agafonov1978]

9.3.5.1.1 Diphtheria-Tetanus-Pertussis (whole cell) Vaccines

Parent du Châtelet *et al* administered three monthly injections of **$DTP_{w(PMSV)}$** to African infants recruited at age 2-to-3 months, using prefilled disposable jet injection cartridges (Imule®) and an investigational Mini-Imojet® needle-free jet injector (JI), and compared it to similar controls receiving the same vaccine and doses by needle-syringe (N-S).[Parent du Châtelet1997] Comparing the JI and N-S groups, frequencies of delayed reactions for pain were 34.2% and 27.4%, respectively (not significant [NS]), 68.3% and 51.2% for induration ($p<0.05$), 1.2% and 2.4% for adenopathy (NS), and 4.9% and 2.4% for fever (NS).

9.3.5.1.2 Hepatitis A Vaccines

Local redness was reported by significantly more soldiers (23%) who received their first dose of Merck **HAV** vaccine IM by Ped-O-Jet® injector, compared to 3% among those vaccinated IM by needle-syringe.[Hoke1995] After the second dose, the significantly-different jet injector/needle-syringe frequencies were 14%/0% for redness, and 8%/0% for swelling. After the third dose, only redness occurred with significantly increased frequency among the jet injector recipients (18%) versus the needle-syringe group (5%). Nevertheless, the investigators concluded that "the jet injector is a highly satisfactory means for mass inoculation of military recruits with hepatitis A vaccine".[Hoke1992]

The Pasteur-Mérieux (now Sanofi-Pasteur) **HAV** vaccine was administered SC by Mini-Imojet® jet injector to 48 adults, and compared to needle-syringe injections by the IM (n=50) and SC (n=49) routes.[Parent du Châtelet1997] Relative frequencies of side effects for SC jet injector, IM needle, and SC needle were 35% / 13% / 26%, respectively, for delayed pain (p=0.05). No significant difference in proportions were found between the three injection groups for delayed erythema (9% / 0% / 13%, respectively), induration (2% / 0% / 0%), hematoma (7% / 0% / 7%), adenopathy (0% / 2% / 2%), and fever (11% / 9% / 9%).

A Biojector® 2000 needle-free jet injector (JI), similar to that proposed to be used in this study, was used to administer two doses IM of GlaxoSmithKline **HAV** vaccine, which was also administered IM by needle-syringe (N-S).[Williams2000] There was no significant difference in the frequencies of systemic side effects between the two study groups. But local reactions were more frequent in the JI group: redness after 58 (20%) of 289 doses (compared to 6 [2%] of 288 N-S doses); swelling after 57 (20%) doses (N-S: 9 [3%]); and bruising after 75 (26%) doses (N-S: 7 [2%]).

9.3.5.1.3 Hepatitis B Vaccines

Three doses of Merck **HBV** vaccine were administered SC in unblinded fashion by Ped-O-Jet® injector above the deltoid insertion region of the non-dominant arms of 19 volunteer adults. These injections were compared with simultaneous saline injection by needle-syringe (N-S) into the dominant arms.[Lemon1983] Induration of >5 mm was reported after 9 (16%) of 57 jet injections, compared to only 1 (2%) of placebo N-S control injections. Erythema of >5 mm reported in 5 (9%) of jet injections, compared to none from N-S placebo. Firm, indurated, painless nodules from 5-to-10 mm in diameter appeared one or two days after 9 (16% of 57) jet injections. All eventually disappeared, at times leaving a pigmented macule.

A Biojector® 2000 device was used to administer GlaxoSmithKline **HBV** vaccine IM to 47 randomly selected adult volunteers, and to 50 vaccinees by needle-syringe.[Matheï1997] Local side effects of soreness, redness, or swelling were reported significantly more frequently (126 adverse effects) after 93 jet injections (doses 1 and 2) than after 98 needle-syringe injections (49 adverse effects).

9.3.5.1.4 Tetanus and Diphtheria Vaccines

Reactogenicity at 3 days to a monovalent tetanus toxoid vaccine adsorbed on aluminum hydroxide (Tetavax®, Pasteur Mérieux Sérums et Vaccins, now Sanofi Pasteur) was examined in 213 African adults vaccinated with the investigational Imule® disposable cartridge and Mini-Imojet® needle-free injector, without the use of a control group receiving needle-syringe injection to compare adverse reactions.[ParentduChâtelet1997] Frequencies of AE were reported for pain (68.5%), fever (35.9%), induration (26.0%), erythema (7.7%), and hematoma (0%).

Brazilian investigators administered via Press-O-Jet™ needle-free injector a dose volume of 1.0 mL of tetanus toxoid precipitated on alum to 300 adults and 350 children in São Paulo, and reported "no disagreeable reactions".[Veronesi1966]

An aluminum-phosphate-adsorbed bivalent **Td** vaccine was administered via needle-free Dermo-Jet® injector in a 0.1 mL intradermal dose to 19 adults, and found by the investigators to be "well-tolerated" and equivalent to an SC injection.[Wegmann1976]

Barrett studied immunization of high-school students with combination **Td-POL$_{IPV}$** vaccine using the American Hypospray® jet injector, finding 12 percent to have a transient local erythema of "3+ severity", defined as a reddened area of 80 square millimeters, with accompanying induration, heat, and tenderness.[Barrett1962]

9.3.5.1.5 Typhoid Vaccines

Agafonov reported on typhoid vaccine via jet injection with a Soviet jet injector, finding the frequency of systemic reactions to the typhoid vaccine was 6.9%, while local reactions were 82% - 93%.[Agafonov1978] Comparing jet injection with needle-syringe for other typhoid and typhoid-paratyphoid vaccines (**TYD_{AKD}** and **$TYD\text{-}PTD_{TAB}$**), Edwards found 82 - 88 percent of jet injection vaccinees had one or more local reactions (pain, erythema, heat, swelling, tenderness, induration, or nodes), compared to only 24 percent of needle-syringe recipients.[Edwards1974] By 72 hours, only 8 percent of the jet-injected **TYD_{AKD}** vaccinees and none of the **$TYD\text{-}PTD_{TAB}$** vaccinees still had such local reactions.

9.4 Intradermal Route of Vaccination

9.4.1 Vaccines other than Influenza

The intradermal (ID) route has been described in the literature for at least 16 different vaccine types. There are numerous reports, of course, for those vaccines in which the ID route is the normal one, such as **BCG**,[Chambon1970a, Chambon1970b, Chambon1970c, Collas1973, Carnus1973, Carnus1974, Fillastre1970] smallpox,[Fenner1988] and combined **BCG**-smallpox vaccine.[Vaughan1972, Vaughan1973] The intradermal route has also been studied with good results for killed vaccines such as typhoid[Tufts1931] and rabies,[Briggs2000] the latter of which has been used widely for dose-sparing purposes in the developing world.[Wilde2005] Generally good results have been reported for hepatitis B,[Bryan1990] but not always,[Coberly1994] while mixed results have been reported for cholera,[McBean1972] hepatitis A,[Brindle1994, Pancharoen2003, Pancharoen2005] and measles.[Calafiore1966, Kok1983, deMoraes1994] Other vaccines studied rarely by this route include polio,[Salk1953a, 1953b] meningococcal A, diphtheria-tetanus-pertussis, tetanus-diphtheria, tick-borne encephalitis, and Rift Valley fever).

In addition, intradermal jet injection has been used to administer immunomodulators like interferon,[Nathan1986] as well as tuberculin (purified protein derivative, PPD) for tuberculosis skin testing among patients of all ages.[Bettag1967, Brólio1976, Cockburn1965, DePartearroyo1966, Dull1968, Hendrix1966, Luby1968, Marsallon1972, Morse1967, Neumann1973, Wijsmuller1975]

9.4.2 Influenza by Intradermal Needle Injection

There is a substantial literature, since the 1930s, starting with Thomas Francis (of Salk polio vaccine trial fame),[Francis1937] documenting the equivalence and occasionally improved immunogenicity of intradermal influenza vaccination by needle-syringe compared to larger doses by the subcutaneous and intramuscular routes.[Bruyn1947, VanGelder1947, Weller1948, Bruyn1949a, Bruyn1949b, Edwards1958, Hilleman1958, Kirkham1958, Sanger1959, Stille1959, Beasley1960, Saslaw1964, Clark1965, Tauraso1969, Marks1971, Brown1977, Halperin1979, Spiegel1994a, Belshe2004, Kenney2004,] On the other hand, a few studies found ID influenza responses less then IM or SC on some or all of the antigens that were studied.[Boger1957, Saslaw1963, Phillips1970, Sigel1975, Hutchinson1977, Herbert1979]

9.4.3 Reduced-dose Influenza Vaccination by Different Routes

When the same reduced dosage of influenza antigen was compared via the intradermal versus IM or SC routes, there were conflicting results from clinical trials. Bruyn *et al* found GMTs in children receiving 0.2 mL intradermally of **INF** to be higher than those receiving the same dose SC,[Bruyn1949a] as did Davies *et al*.[Davies1969] and Tauraso *et al*[Tauraso1969] administering 0.1 mL by both routes. Stille *et al* also found greater ID responses when administering small antigen masses, but conversely, SC responses exceeded ID ones when delivering larger doses.[Stille1959] On the other hand, McCarroll *et al*,[McCarroll1958] studying hospital employees 18 to 65 years of age, and Klein *et al*,[Klein1961] studying infants 2 months to 5 years of age, both found no difference in responses between the ID and SC routes.

Regarding systemic reactions, among 101 infants from 2 months to 2 years of age receiving 0.1 mL of influenza vaccine in the Klein *et al* study, febrile reactions were reported among 34.7% (17/49) in the intradermal group and only 19.2% (10/52) in the subcutaneous group getting the same reduced dose.[Klein1961] Similarly, local reactions of small areas of erythema and induration with 2 to 3 days of slight tenderness and itching were described for "all" intradermal participants (ages 2 month to 5 years, n = 96), while only 2 of 94 children vaccinated subcutaneously had local pain and induration.

9.4.4 Influenza by Intradermal Needle-free Jet Injection

The use of needle-free jet injectors to administer influenza vaccine intradermally (ID) has been studied and documented since the 1940s.[Parker1948] Two studies of jet injected ID influenza vaccination in particular were performed. Davies *et al* studied monovalent A_2/Australia/54 and found significantly higher GMTs for 0.1 mL administered ID by JI (114) than for 0.5 mL by the SC route (75.8).[Davies1969] Although there was no statistically significant difference between the proportion with a 4-fold titer rise in the JI ID group (58%) versus the full-dose SC one (40%). On the other hand, Payler *et al* studied a trivalent product, administering 1.0 mL SC compared to 0.15 mL ID by JI, and found a trend to SC to be superior, but the difference did not reach statistical significance.[Payler1974]

9.5 Influenza Disease Burden

In 2004, recognizing the morbidity and mortality of influenza disease in young children, the Advisory Committee on Immunization Practices (ACIP) of the CDC first recommended universal vaccination of all children from the ages of 6 through 23 months, healthy and otherwise,[CDC2004] and continues to recommend so in 2005.[CDC2005] Similarly, the Pan American Health Organization encourages countries to introduce yearly seasonal influenza vaccination in routine programs for children from 6-23 months of age.[PAHO2005]

Influenza causes both epidemic and pandemic disease with the 1918-19 pandemic as the most devastating, with an estimated 40-50 million deaths worldwide.[Nicholson2003] In the United States, influenza is associated with an average of 36,000 underlying respiratory and circulatory deaths annually and more than 200,000 hospitalizations.[Thompson2003]

Each year between 5% and 20% of the population is infected with influenza.[Monto1986] Based on the 1980 population, among persons age <20 years, the annual burden of influenza in the United States includes an average of:

- 13.8 to 16.0 million excess influenza-related illnesses
- 152.0 to 176.4 million excess influenza-related illness days
- 47.1 to 54.7 million excess influenza-related excess bed and restricted activity days.

Among persons age ≥20 years, the annual burden of influenza in the United States is on average:

- 4.1 to 4.4 million excess influenza-related illnesses
- 65.7 to 70.6 million excess influenza related illness days
- 16.6 to 17.9 million excess influenza-related excess bed and restricted activity days [Sullivan1993]

About 90% of influenza-associated deaths occur among persons aged 65 years and older. The influenza-attributable mortality rate for persons aged 85 years and up is significantly higher as compared to persons 65 to 69 years.[Thompson 2003]

Table 1

Estimated Annual Influenza Associated Mortality 1990-91 Through 1998-99 Seasons [1]

Age Group	Rate per 100,00 person-years
<1	0.6
1-4	0.4
5-49	0.5
50-64	7.5
≥65	98.3
Total	13.8

[1] Underlying respiratory and circulatory deaths
Source: Unpublished, CDC
(http://inside.nip.cdc.gov/divisions/isd/irl/dis_ep_burd.asp)

Influenza A (H3N2) viruses are associated with the highest annual rates of influenza-associated hospitalizations. Persons aged 65 and up have the highest rates of influenza-associated hospitalization followed by children younger than 5.

On average 94,735 primary pneumonia and influenza hospitalizations, 133,900 any listed pneumonia and influenza hospitalizations, and 226,054 primary respiratory and circulatory hospitalizations per year occur in the US.[Thompson 2004]

Table 2

Age-Specific Annual Average Numbers and Rates of Influenza-Associated Hospitalizations [1]

Age Groups	Number	Rate
<5	20,031	107.9
5-49	34,867	20.8
50-64	29,447	83.8
65-69	18,301	189.7
70-74	26,501	321.2
75-79	27,516	431.1
80-84	28,578	686.1
≥85	40,813	1194.9
Total	226,054	88.4

[1] Respiratory and circulatory hospitalizations, primary
Source: Unpublished, CDC (http://inside.nip.cdc.gov/divisions/isd/irl/dis_ep_burd.asp)
ACIP 2005 Recommendations[CDC2005]

9.5.1 Epidemiology of Influenza in Dominican Republic and Caribbean

The epidemiology of influenza in the Dominican Republic is atypical in that this Caribbean island experiences transmission more-or-less year round as a consequence of virus introductions from tourists escaping winters in North America from October through March, and winters in South America from April through September (personal communication, Dr. Jesús Feris Iglesias, 2005). There is scant quantitative data available, however, on influenza incidence from the Dominican Republic or from its neighbor, Haiti, on the island of Hispaniola.

General health indicators for the Dominican Republic are summarized by PAHO.[PAHO2004] The estimated infant (0-12 months) mortality rate between 1995 and 2000 was 40 per 1,000 live births (l.b.). In 1999, the leading cause of morbidity in such infants was acute respiratory infection (668.8 per 1,000 l.b.), followed by acute diarrheal diseases (329.3), and parasitoses (138.5). In children 1-4 years, respiratory infections were also the leading cause of illness at 221.2 cases per 1,000 population, followed by acute diarrheal diseases (69.4). The immunization infrastructure performed well, with the proportion of infants less than one year of age immunized with **POL_{OPV}**, **DTP**, and **TUB_{BCG}** measured at 87%, 72%, and 96%, respectively. Influenza vaccine is not a routine antigen included in the public immunization program in the Dominican Republic.

Puerto Rico, another island about 100 km to the east, does have somewhat more epidemiologic data on influenza, which may be relevant to the Dominican Republic because of geographic, climatic, and cultural similarities. Among Puerto Rican children less than 6 years of age, the rate of influenza incidence in the selected years 1987, 1989, 1992, 1994, and 1996 was 8.1, 38.1, 12.7, 6.6, and 2.4, respectively, per 100 persons, suggesting substantial morbidity and great year-to-year variation.[PuertoRico2005]

Among the English-speaking Caribbean members of the Caribbean Epidemiology Center (CAREC), which does not include the Dominican Republic, acute respiratory infections decreased over time, but still remain a leading cause of death in children 1-4 years of age, with an age-adjusted rate of 30 per 100,000 in 1980 and 5 per 100,000 in 1995.[Holder2000] In 2000 and 2001, a total of 71,201 and 60,236 influenza cases, respectively, were reported from the 20 CAREC member countries.

On Jamaica, an island 400 km to the southwest of the Dominican Republic, a study of etiologic agents of acute respiratory infections determined the proportion in 83 malnourished children.[Christie1990] Influenza virus was isolated from 14% (12 of 83), second only to parainfluenza viruses (18%), and more frequent than adenovirus (12%), RSV in 8%, and Mycoplasma in 8%. During the 1918-19 influenza pandemic, the virus was noted to have swept through the Caribbean and resulted in an estimated 100,000 deaths.[Killingray1994]

9.6 Objectives and Rationale for Study

9.6.1 Study Objectives

This study intends to demonstrate a dose-sparing intradermal (ID) method to allow larger numbers of young children to be protected when supplies of influenza vaccine are limited, and to prove the principle that needle-free jet injection can obviate the cost, time, expertise, and difficulty administering ID injections by the Mantoux needle-syringe method. DCJIs thus also avoid this and other drawbacks and dangers of needle-syringe injections, such as safe sharps disposal, unsterile reuse, and needlestick injury.

9.6.2 Rationale

This study will provide new clinical data on the degree of safety and immunogenicity of protecting young children from influenza by a needle-free reduced-dose route. It would provide information essential for developing country public health officials, immunization programs, and clinicians who must make difficult policy decisions as they face likely vaccine shortages in the inevitable pandemic of influenza of the future.

There is already a large body of data on the immunogenicity and reactogenicity of intradermal administration of **INF** vaccine by needle-syringe (see sections 9.4.2 and 9.4.3), and prior studies of this route using needle-free jet injection in adults[Davies1969] and in schoolchildren[Payler1974] (see section 9.4.4). But there is none in the intended age group of 6-to-24 month old children. This study will fill that gap for an age group at high risk for morbidity and death from influenza disease.

9.6.3 Intended / Potential Use of Study Findings

Proving the concept of dose-sparing by intradermal (ID) injection could provide a useful strategy for public health policymakers in developing countries to protect their

populations against pandemic influenza threats for which vaccine supplies are likely to be in short supply. Demonstrating that needle-free jet injectors can accomplish this ID delivery quickly and easily without the risks of needlestick accidents can provide a valuable logistical tool for mass campaigns.

Although the particular intradermal spacer to be utilized in this study is a proprietary design of the jet injector manufacturer, the concept of creating a gap between a jet injector nozzle and the skin to achieve intradermal delivery by weakening a standard perpendicular jet stream may be considered *prior art* and thus no longer patent-protectable. It has been long described in the scientific literature and applied in the field with various jet injectors (section 9.3.1).[Meyer1964, Kalabus1967, Dull1968, Zsigmond1999a, Zsigmond1999b, Sugibayashi2000, Med-E-Jet2006] This precedent provides "freedom to operate" for various manufacturers to adapt and study their devices in achieving ID delivery. Thus, the findings of this study may spur further competitive research and development to pursue a safer, needle-free administration route for influenza and perhaps other antigens.

9.6.4 Research Questions

1. Will the investigational study arm of children receiving two doses of 0.1 mL of influenza vaccine administered ID demonstrate *non-inferiority* to the immune responses achieved by the control study arm of children receiving two conventional, full-doses of 0.25 mL administered IM by needle-syringe? (Primary endpoint: proportion of participants achieving inverse titer of ≥40 on hemagglutination inhibition assay for each virus strain in the vaccine.)

2. What will be the safety profile of jet-injected, reduced-dose, intradermal influenza vaccination (erythema, induration, limb swelling, bleeding, and other local and systemic reactions) delivered by DCJI using an investigational spacer for ID delivery?

3. Will an investigational study arm of children receiving two reduced doses of 0.1 mL of vaccine administered IM by needle-syringe demonstrate *non-inferiority* to the immune responses achieved by the control arm of children receiving conventional, full-dose 0.25 mL administered by the same method in the same target tissue (obviating any need for intradermal delivery to achieve dose sparing)?

10. Investigational Plan

10.1 Overall Study Design and Plan

The study is a randomized, double-blinded, controlled, phases I and II clinical vaccine trial of safety and immunogenicity, among children enrolled at ≥9 -to- <24 months of age. It will compare using a *non-inferiority* statistical model two standard doses in this age group of

0.25 mL of a commercial, trivalent, inactivated, split-virus product administered intramuscularly (IM) one month apart by conventional needle-syringe injection with two reduced doses of 0.1 mL, administered by either the intradermal (ID) route via needle-free jet injector or by the IM route with needle-syringe. The ID route will utilize an investigational ID spacer on a U.S.-licensed needle-free jet injector.

Healthy participants will be recruited from among eligible patients and their siblings attending immunization, outpatient, or inpatient clinics and wards of a large, public tertiary care children's hospital. Participants will be randomly assigned to receive two doses of Sanofi-Pasteur Vaxigrip® influenza vaccine by one of the three study arms. At the conclusion of the study, at the time of collection of the final blood specimen, participants in the two investigational groups that received reduced doses will receive a third "insurance" dose of the influenza vaccine via the conventional route, method, and dose, to ensure adequate protection. Six months after this study "graduation", all participants will receive a fourth "bonus" booster dose for protection during the following influenza season.

10.1.1 Study Arms

The three study arms are:

- Group "**IM-NS-0.25**" (controls) - two full 0.25 mL doses administered intramuscularly (IM) by standard needle-syringe (NS)
- Group "**ID-JI-0.1**" (investigational) - two reduced 0.1 mL doses administered intradermally (ID) by needle-free jet injector (JI)
- Group "**IM-NS-0.1**" (investigational) - two reduced 0.1 mL doses administered intramuscularly (IM) by needle-syringe (NS)

10.1.2 Phases

The studies will be conducted in two phases:

Phase I. 48 children assigned randomly into 3 study arms of 16 each.

Phase II. Upon analysis of adverse events and clearance by the DSMB, an additional 402 infants of the same age range will similarly be enrolled into the same three study arms of 134 participants each.

Total participants in phase I and II = 450 (150 in each of three study arms).

If phase II is not pursued for whatever reason, sera from phase I will be assayed as described below, after which the study group codes will be unblinded for analysis of phase I by the investigators.

10.1.3 Endpoints

Study endpoints to be measured or determined are the following:

10.1.3.1 Primary Immunologic

The primary immunologic endpoint of the study will be the titers measured by hemagglutination inhibition on serum drawn on the days of vaccine dose 1, vaccine dose 2, and one month after dose 2. See section 10.5.1 for definitions of immunogenicity variables *seroconversion*, *seroprotection*, *mean geometric increase*, etc.

10.1.3.2 Primary Safety

Primary safety endpoints are local and systemic reactions manifested for each of the following prompted adverse events during the intervals specified (see section 10.5.2.2).

Immediately after injection:

Local at injection site

- **Drop of blood** on skin
- **Flow of blood** on skin
- **Wetness** (not blood) on skin
- **Other**, may be entered and specified

≥30 -to- ≤60 minutes after injection:

Local at injection site

- **Erythema**, largest diameter
- **Swelling**, largest diameter
- **Induration**, largest diameter
- **Other**, may be entered and specified

Systemic

- **Fever**, via axillary temperature
- **Sleepiness**
- **Irritability**
- **Seizures**
- **Anaphylactic shock**
- **Other**, may be entered and specified

0-2 days and 3-7 days after injection:

Local at injection site

- **Tenderness**, 0 to 4 scale
- **Redness**, largest diameter
- **Swelling**, largest diameter
- **Hematoma**, largest diameter
- **Midpoint circumference** of limb (compared to baseline)

- **Entire limb swelling**
- **Other**, may be entered and specified

Systemic

- **Fever**, via axillary temperature
- **Antipyretic medication given**
- **Change in eating habits, loss of appetite**
- **Sleepiness**
- **Irritability**
- **Unusual or inconsolable crying**
- **Vomiting**
- **Diarrhea**
- **Seizures**
- **Other**, may be entered and specified

8-28 days after injection:

Local at injection site or *systemic*

- **Unprompted adverse events** solicited on diary card and reported by any means

10.2 Physical Facilities

10.2.1 Description

Clinical aspects of the study will take place at the *Hospital Infantil Dr. Robert Reid Cabral* (HIRRC), Avenida Abraham Lincoln No. 2, Centro de los Heroes, in the city of Santo Domingo [Distrito Nacional], capital of the Dominican Republic. This is the national children's hospital, which is a component of the *Secretaría del Estado de Salud Pública y Asistencia Social* (SESPAS, equivalent to a ministry of health). HIRRC serves as a major primary, secondary, and tertiary treatment facility and also provides public health outpatient immunization services for patients both from within its catchment area in and around the capital district, as well as other parts of the country. It has prior experience in the conduct of infectious disease surveillance[Gomez1998, Gomez2000, Terrero1998, DEI1995, Rondón1998, FDI2001] and of clinical vaccine trials.[Fernandez1999, Fernandez2000a, Fernandez2000b]

The HIRRC has 350 inpatient beds and admits approximately 1,000 pediatric inpatients per month (12,000/year) for stays averaging seven days. The most common causes for admission are pneumonia and septicemia. Since 1963, the HIRRC has served as a formal residency training facility in the fields of pediatrics, pediatric infectious diseases, pediatric cardiology, and pediatric surgery.

The HIRRC Department of Infectious Diseases (DEI in Spanish), which will serve as the base for the proposed study, has 42 beds in its dedicated ward, and is served by a Chief of Service and five pediatric residents.

The HIRRC laboratory possesses refrigerators to maintain the cold chain for the **INF** vaccine, and a –70º C freezer for storage of sera. Both will be backed up with a ≥20 kilo-watt emergency power generator to be installed and tested onsite prior to commencement of the study, under financing arranged under contract with CDC. The HIRRC laboratory has trained personnel and equipment to centrifuge blood samples, and prepare and ship aliquots of sera.

10.2.2 Participant Recruitment

Eligible participants for this study will be recruited from among children present in the hospital's Healthy Children and Vaccination Service, known informally as the immunization clinic (C*entro de Vacunas*).

The HIRRC immunization clinic is located in a satellite building on the ground level just steps from the main outpatient building, and draws a heterogeneous patient population because it is known for having a consistent supply of vaccines. Many parents bring children long distances to receive immunization services in this popular facility. A review of those receiving measles vaccine at the immunization clinic (when the recommended age was 9 months) in the full six months from February through July 2003, identified a total of 718 patients of age ≥9 -to- <12 months in which both date of birth and age of vaccination were recorded in the medical record (personal communication, Dr. Virgen Gómez, 2003). This represents an average of 120 patients per month, or 28 per week, in an age range that is only one-sixth of the target age range for recruitment into this study of ≥6 –to- <24 months.

Parents of potential participants in the waiting room of the *Centro de Vacunas* will be solicited for interest in the study and invited to learn more at the *Centro del Estudio* [Study Center]. See section 10.6.2.

10.2.3 Study Center

A suite of two rooms (office and injection room) is renovated on the 2nd floor of the main outpatient building for use as the planned research study center (*Centro del Estudio*) for this trial. Chairs in a waiting area are located immediately outside the suite. The physician investigator's office where medical history and clinical examinations will be performed is located about 15 meters down the hall. In these rooms, the orientation, interview, and explanation of the informed consent will be conducted with the parents, and the child either recruited at that time, dismissed as ineligible, or scheduled to return at a later date upon reaching eligibility. Vaccinations and return followup visits will occur in the Study Center.

10.3 Selection of Study Population

10.3.1 Inclusion Criteria

Participants enrolled in the study will be those who meet the following criteria from among infants presenting at the Study Center according to the recruitment methods described in section 10.2:

a. Age from ≥6 -to- <24 months (not having reached 2nd birthday). (The date of first study vaccination may be the same day of the 6th month after the child's day and month of birth, or a later day, but no earlier. Maximum age may **not** include the child's 2nd birthday, nor a later day. For example, a child born on 1 January 2005 may only be vaccinated for dose 1 from 1 June 2005 through 31 December 2006. A child born on 31 May 2005 may only be enrolled from 1 December 2005 (not 30 November) through 30 May 2007.)

b. Born after a full-term pregnancy of gestational age of ≥37 weeks, and a birth weight of ≥2.5 Kg (≥5 pounds, 8 ounces)

c. History of prior or first attendance as a patient, or as a sibling of a patient, seeking routine immunization or other clinical care at the HIRRC

d. The accompanying parent(s) or legal guardian(s) provide written informed consent after the nature of the study has been explained, and agree to bring the infant back to the clinic for all visits scheduled in the study

e. Up-to-date for routine doses of vaccines officially recommended for the participant's age in the Dominican Republic to prevent tuberculosis, polio, diphtheria, tetanus, pertussis, hepatitis B, and *Haemophilus influenzae B* (See exclusion criterion below for any vaccination received within 30 days prior to first study vaccination.)

f. In good health, as determined by medical history and physical examination collected in accordance with the Case Report Form (CRF), and by the clinical judgment of the investigators.

10.3.2 Exclusion Criteria

To be excluded are infants WHOSE PARENT(S)/LEGAL GUARDIAN(S) … :

a. are unable or unwilling to give written informed consent for their infant to participate in the study

b. cannot be contacted by telephone (family's own or a neighbor's) by the study nurse if necessary for surveillance of adverse events if scheduled followup return appointments are not fulfilled

c. are unable to complete the diary form for adverse events, to measure and record temperature with the TraxIt™ skin-appliqué thermometer, to measure the maximal diameter of local reactions or limb circumference, or have difficulty reading or understanding written instructions, or other factors which indicate exclusion in the judgment of the study staff

Also to be excluded are INFANTS who … :

d. have fever (by parental report or by rectal temperature ≥38.5° C or axillary ≥38.0° C) currently or within the past 3 days, or who are currently suffering from an acute or chronic infectious disease (including known HIV)

e. have had an acute or chronic infection requiring systemic antimicrobial therapy (antibiotic or antiviral) or other prescribed treatment within the past 21 days. This includes any underlying illness that may limit their response to vaccination, such as those receiving intravenous immunoglobulin for agammaglobulinemia, or systemic steroid therapy

f. are malnourished, defined by weight less than two standard deviations below the median weight for their age

g. are allergic to eggs, or have a history of any anaphylactic shock, asthma, urticaria, or other allergic reaction after previous vaccinations, or have allergy or hypersensitivity to any component of the study vaccine

h. have ever received previously any influenza vaccine

i. have received within the prior 28 days, or for whom there is the indication to receive in the next 56 days, any non-study vaccination or investigational agent outside of the study (This would exclude infants "behind schedule" and needing to receive on the day of first interview protection against tuberculosis, polio, diphtheria, tetanus, pertussis, hepatitis B, *Haemophilus influenzae* type b, measles, mumps, or rubella. See section 10.4.7 for the effect on recruited age ranges)

j. have a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time

k. have currently any serious confirmed or suspected disease, such as metabolic, cardiac, or autoimmune disease, or diabetes

l. have a history of epilepsy or a seizure disorder, or neurodevelopmental disorders such as autism

m. have a genetic anomaly or known cytogenic disorder (e.g., Down's syndrome)

n. have leukemia, lymphoma, or any other cancer/neoplasm

o. have known or suspected immune dysfunction, including HIV infection, or receives now or in the past immunosuppressive therapy, including the use of steroids associated with the suppression of the hypothalamic-pituitary-adrenal (HPA) axis (systemic corticosteroids, e.g., 1 mg/Kg/day of prednisone or its equivalent, or the chronic use of inhaled high-potency corticosteroids, e.g., 800 µg per day of budesonida or 750 µg per day of fluticasone)

p. have ever received blood, blood products, or parenteral preparations of immunoglobulin

q. have any other serious disease (e.g., with signs of cardiac or renal failure), including progressive neurologic disease

r. have any condition which, in the opinion of the investigator, may interfere in the evaluation of the objectives of the study

10.3.3 Removal of Participants from Therapy or Assessment

The parent(s) or legal guardian(s) of infant participants may withdraw consent for his/her/their child to participate in the study at any time without prejudice. At any time, the investigator may withdraw a participant if, in his or her clinical judgment, it is in the best interest of the participant or if the participant or parent(s) cannot comply with the protocol. Whenever possible, the tests and evaluations listed for the final visit should be carried out. The Dominican and U.S. Senior Investigators should be notified in a timely manner to discuss all study withdrawals.

10.4 Treatments

10.4.1 Treatments Administered

All recruited participants will receive the same influenza vaccine (**INF**), but in different antigen volumes, tissue compartments, or means of injection.

- Group "**IM-NS-0.25**" (controls) - two full 0.25 mL vaccine doses administered one month apart intramuscularly (IM) by sterile needle of 22-25 gauge, with a minimum length of 25mm (1-inch), attached to a sterile disposable needle.

- Group "**ID-JI-0.1**" (investigational) - two reduced 0.1 mL vaccine doses administered one month apart intradermally (ID) by sterile, disposable needle-free Biojector® Syringe [cartridge] #2 and Biojector® 2000 jet injector, fitted with an investigational spacer on the cartridge nozzle creating a gap of 2 cm between nozzle orifice and skin.

- Group "**IM-NS-0.1**" (investigational) - two reduced 0.1 mL vaccine doses administered one month apart intramuscularly (IM) by sterile needle of 22-25 gauge, with a minimum length of 25mm (1-inch), attached to a sterile disposable needle intramuscularly (IM) by needle-syringe (NS)

One month after the 2nd dose of INF vaccine described above, participants in the two investigational groups (**ID-JI-0.1** and **IM-NS-0.1**) that received reduced doses will receive a 3rd "insurance" dose of 0.25 mL of the same vaccine intramuscularly (IM) by sterile needle-syringe. Controls (**IM-NS-0.25**) who already received the standard two full doses will receive a mock injection to avoid extravaccination. Six months later, all groups three groups will return for a "bonus" booster dose.

Figure 4. Vaxigrip® vaccine 5 mL vial (bilingual English-French label)

10.4.2 Identity of Investigational Vaccine

(Vaxigrip®, Sanofi-Pasteur)

The Vaxigrip® brand of Inactivated Influenza Vaccine Trivalent Types A and B (Split Virion) to be used in this study (see Figure 4) is manufactured in Val De Reuil, France, by Sanofi-Pasteur (headquartered in Lyon, France and initially known as Institut Mérieux, then as Pasteur Mérieux Sérums et Vaccins, Pasteur-Mérieux-Connaught, and later Aventis Pasteur). The Vaxigrip® brand of influenza vaccine was first marketed in France in 1968. [Saliou2005] Current formulations were developed in the 1980s and first marketed in 1995. It uses octoxynol-9 (Triton® X-100) detergent to "split" the virus particles and render them less reactogenic, a technique that previously required an ether compound that was dangerous to handle in manufacturing. [Lina2000]

Vaxigrip® is currently licensed for sale and routine use of its prefilled syringe packaging format by the Dominican Republic national pharmaceutical regulatory authority, the General Directorate of Drugs and Pharmacies (Dirección General de Drogas y Farmacias), a unit of the Secretariat of State of Public Health and Social Assistance (SESPAS [ministry of health]). However, the Vaxigrip® vaccine packaged in standard, multidose 5 mL vials to be used in this study are not specifically registered in the Dominican Republic, and thus will be imported and administered under special investigational authorization from the national regulatory authority.

10.4.2.1 Product Characteristics

As described in its Canadian product [insert] monograph: [AventisPasteur2005]

> "VAXIGRIP® [Inactivated Influenza Vaccine Trivalent Types A and B (Split Virion)] for intramuscular use, is a sterile suspension prepared from influenza viruses propagated in chicken embryos. Following incubation, the virus-containing fluids are collected and clarified and the viruses are concentrated then purified by zonal centrifugation using a sucrose density gradient. Subsequent stages consist of treatment with octoxynol-9 (Triton® X-100) to obtain split antigens, then inactivation using formaldehyde solution. The split-antigen is suspended in sodium phosphate-buffered, isotonic sodium chloride solution. The type and amount of viral antigens contained in VAXIGRIP® conform to the current requirements of the World Health Organization (WHO)." [WHO2005]

The study will commence with the 2006 Southern Hemisphere formulation of Vaxigrip® **INF** vaccine, the content of which is summarized in **Table 3**. This formulation becomes available in early 2006 and will expire on 31 December of 2006. It is anticipated that a proportion of vaccinations will occur after that date (see timeline in section 5), requiring use of either or both of the next available formulations (2006-2007 Northern Hemisphere and 2007 Southern Hemisphere). Their content will not be known until after the study begins. If there is a change of strains between formulations used, each participant will receive all his or her doses from the same unexpired formulation.

As described in section 9.5.1, the biseasonal epidemiology of influenza in the Dominican Republic justifies use of either Northern or Southern hemisphere formulations. If strains change between seasonal formulations used in the study, serologic assays with appropriate antigens will be segregated accordingly.

Table 3 Vaxigrip® **INF** vaccine content, by study arm

2006 Southern Hemisphere formulation	Study Arm	
	"ID-JI-0.1" "IM-NS-0.1"	"IM-NS-0.25" (control)
Hemagglutinin antigen		
A/New Caledonia/20/99 (H1N1) [IVR-116]:	3 µg	7.5 µg
A/New York/55/2004 (H3N2) [NYMC X-157] (A/California/7/2004-like):	3 µg	7.5 µg
B/Victoria/2/87 lineage (B/Malaysia/2506/2004-like):	3 µg	7.5 µg
Sodium-phosphate buffered, isotonic sodium chloride solution	up to 0.1 mL	up to 0.25 mL
Formaldehyde	≤6 µg	≤15 µg
Thimerosal preservative	0.4 µg	1 µg
Triton® X-100, sucrose, neomycin	Trace	trace

10.4.2.2 Storage

As per the manufacturer's recommendations, [Aventis-Pasteur2004] the vaccine should be stored at +2° C to +8° C when not being used. It must NOT be frozen. Open only one vial at a time. The doses for all three study arms should be filled from the same vial until empty. When not vaccinating participants, return the vial to the cold box carrier or vaccine refrigerator. At the end of the clinic day, return the vial from the cold box carrier to the vaccine refrigerator for overnight storage. Dispose of any vial, whether empty or not, which has been entered and in use for 7 days, or for which there is any other evidence or suspicion that it has been compromised.

10.4.2.3 Directions for Vaccine Administration

Preparation. [Aventis-Pasteur2004]

- Inspect the vaccine vial for extraneous particulate matter and/or discoloration before use. If these conditions exist, the product should not be administered. Do not remove either the vial stopper or the metal seal holding it in place. Aseptic technique must be used for withdrawal of each dose.
- Remove the aluminum tab covering the vial stopper, and swab the stopper with alcohol.
- Open and attach to the vaccine vial a sterile, disposable multidose vial adaptor (e.g., SmartSite® vial access device - http://www.alarismed.com/na/products/smartsite_compon.asp) by pushing its plastic spike through the vial stopper.
- Write onto the vial label the date when the vial adaptor is attached.

Needle and syringe administration for study groups *IM-NS-0.1* and *IM-NS-0.25*

- Politely request the parent(s) and any other personnel to remain blinded by study protocol to leave the injection room. Confirm the participant has been assigned to the intramuscular, needle-syringe, reduced-dose study arm (**IM-**

NS-0.1) or to the intramuscular, needle-syringe, full-dose study arm (**IM-NS-0.25**).

- Swab the vial adaptor port valve with alcohol.
- Gently shake the vial to uniformly distribute the suspension before withdrawing each dose.
- Holding the vial upside down, insert upward into the port the Luer tip of a sterile, 1mL capacity tuberculin-style or universal vaccination syringe.
- Draw downward on the syringe plunger until the correct volume of either 0.1 mL or 0.25 mL shows on the syringe (according to the participant's assigned study group), then withdraw an additional amount (~0.1 mL) to account for wastespace in the syringe tip and needle hub.
- Maintaining the syringe pointing upward, remove it from the vial adapter and attach a sterile 22-25 gauge needle with a minimum length of 25mm (1-inch).
- Still holding the syringe upward, pull down on the plunger until at least 0.5 mL of air enters the syringe above the vaccine.
- Tap the syringe barrel until all vaccine liquid has moved to the bottom of the barrel towards the plunger and all air bubbles have risen to the top towards the needle.
- Slowly push up on the plunger until the air is expelled from the syringe and needle and the plunger seal reaches the appropriate dose mark on the barrel (0.25 or 0.1 mL, according to participant group).
- For children aged >1 year, insert the needle intramuscularly to its hub at a 90º angle into the participant's *vastus lateralis* muscle on the anterolateral portion of the mid-to-upper **LEFT** thigh. For children ≥1 year of age, administer into the deltoid muscle of the **LEFT** arm.
- After the injection, observe 5 seconds for any leakage of liquid or blood on the skin.
- Note observations on the case report form, along with vaccine lot number and clock time.
- Cover the injection site with an adhesive bandage (to be removed in 30-60 minutes for inspection).
- Safely dispose of the needle-syringe before the parent enters the room.
- Return the vaccine vial to the cold box or refrigerator unless the next participant will be vaccinated shortly.

Jet injection administration for study group *ID-JI-0.1*

- Politely request the parent(s) and any other personnel to remain blinded by study protocol to leave the injection room. Confirm the participant has been assigned to the intradermal, jet-injected, reduced-dose study group (**ID-JI-0.1**).
- Gently shake the vial to uniformly distribute the suspension before withdrawing each dose.
- Swab the vial adaptor port valve with alcohol.
- Insert into the port the Luer tip of a sterile Biojector® cartridge (#2/green).

- Holding the vial upside down, draw Vaxigrip® vaccine into the cartridge until the correct volume of 0.1 mL shows on the cartridge. Then withdraw an additional amount (~0.1 mL) to account for wastespace in the cartridge nozzle.
- Maintaining the cartridge pointing upward, remove it from the vial adapter.
- Still holding the cartridge pointing upward, pull down on the plunger until at least 0.5 mL of air enters the cartridge above the vaccine.
- Tap the cartridge barrel until all vaccine liquid has moved to the bottom of the barrel towards the plunger and all air bubbles have risen to the top towards the nozzle.
- Slowly push up on the plunger until the air is expelled from the cartridge and the top edge of the orange plunger seal reaches the 0.1 mL dose mark on the barrel.
- Attach a sterile, disposable 2-centimeter spacer onto the cartridge nozzle.
- Verify from the indicator gauge on the Biojector® 2000 jet injector that it is in the green range indicating sufficient pressure of CO_2 gas for the injection. If not, release any remaining residual pressure by slowly unscrewing the CO_2 canister knob, and replace the canister with a new one. Then repeat this step.
- Keeping one's fingers away from the trigger, insert the cartridge into the jet injector front end and rotate all the way clockwise until the indicator window shows green.
- When ready to inject children aged <1 year, hold the spacer, cartridge, and device firmly against the *vastus lateralis* muscle of the anterolateral aspect of the mid-to-upper **LEFT** thigh. Stabilize the back of the infant's thigh with one's other hand. While maintaining a 90º angle between the axis of the cartridge and the thigh, pull and immediately release the injector activator lever and maintain the relative positions of the injector and thigh for one full second to be sure the injection is over. For children ≥1 year of age, follow the above procedure to administer the dose into the deltoid muscle of the **LEFT** arm.
- After the injection, observe 5 seconds for any leakage of liquid or blood on the skin.
- Note observations on the case report form, along with vaccine lot number and clock time.
- Cover the injection site with an adhesive bandage (to be removed in 30-60 minutes for inspection).
- Safely dispose of the cartridge and spacer before the parent enters the room.
- Return the vaccine vial to the cold box or refrigerator unless the next participant will be vaccinated shortly.

10.4.2.4 Vaccine Immunogenicity Profile

Substantial clinical experience in use of the current "Triton® X-100 split" formulation of Vaxigrip® influenza vaccine has accumulated since the early 1990s when it replaced the earlier "Tween-ether split" product, first introduced in France

in 1968. [Lina2000, Saliou2005] Hundreds of millions of doses of this current Vaxigrip® formulation have been distributed and used worldwide over many seasons with, of course, the annual modifications of virus strain composition.

The immunogenicity of Vaxigrip® has been demonstrated in clinical trials in adults from age 18 to 60 years, in the elderly over 60 years, and in children from 6-36 months and from 3-10 years. Annual studies in adults are performed to verify the immunogenicity of annual changes in strain composition (data on file at Aventis Pasteur S.A., Lyon France [AventisPasteur2005]) In the unpublished *Annual Study* (see Figure 5), and in *Study 3 (35),* [Lina2000] a single dose was given and antibody titers were assessed immediately before vaccination and then 21 days later. In *Study 2 (36)* [Gonzalez2000] antibody titers were assessed immediately before the first dose and 27-33 days following the second vaccine dose.

Figure 5. Table 3 from the Vaxigrip® monograph [AventisPasteur2005]

references: (13) = "data on file", (35) = Lina2000, (36) = Gonzalez2000

Table 3: Summary of Patient Demographics for Clinical Trials

Study #	Trial design	Dosage, route of administration and duration	Study subjects (n=number)	Mean age (Range)	Gender
Annual Study (13)	Open	0.5 mL IM	>50 >50	18-60 years >60 years	--
Study 2 (13) (36)	Open	0.25 mL IM; 2 doses 1 month apart	65	6 months to 3 years	Male 37 Female 28
Study 3 (35)	Open	0.5 mL	42 (12 had received prior influenza vaccination)	8-10 years	Male 19, Female 23

The results of these studies are quoted verbatim from the product monograph [AventisPasteur2005]:

> "The efficacy of influenza vaccine is assessed using a surrogate for protection defined as the immune response elicited by the vaccine (hemagglutination inhibition). In the annual studies, the serologic responses of both adult age groups to all antigens must meet the assessment criteria as defined in the European Requirements for Influenza Vaccines (i.e., for subjects 18-60 years – at least one of seroconversion or significant increase in antihemagglutinin antibody titre in >40%, mean GMT increase >2.5, proportion of subjects achieving HI titre or seroprotection >70%, and for

> subjects >60 years at least one of seroconversion or significant increase in antihemagglutinin antibody titre in >30%, mean GMT increase >2.0, proportion of subjects achieving HI titre >60%.) [CPMP1997] Elderly subjects generally respond less well to influenza vaccines than young healthy adults, and those with chronic debilitating medical conditions generally respond less well than healthy subjects of similar age. [Fukuda2004]
>
> "The results in children met the criteria defined for young adults; no criteria for children have been set."

Gonzalez, *et al*, studied two 0.25 mL doses of Vaxigrip® administered one month apart to 67 children from 6 months to 3 years (36 months) of age in an uncontrolled study. [Gonzalez2000] Seroconversion (see definition in section 10.5.1.2) was achieved by 83.7% of the children to the A/H3N2 strain, by 81.6% to the A/H1N1 strain, and by 61.2% to the B strain. Seroprotection (see definition in section 10.5.1.3) rates were 91.8%, 81.6%, and 93.9%, respectively. The geometric mean increases (section 10.5.1.3) were 9.8, 13.3, and 4.1, respectively.

In a recent unpublished poster presentation, [Saliou2005] manufacturer representatives summarized the data from manufacturer trials designated GRT04, GRT11 and GRT51 among children from 6 months to 3 years of age. Precise seroconversion and seroprotection rates were estimated from histogram bars without value labels by interpolating from the scale. The mean seroconversion rates were estimated at 84% for A/H3N2, 88% for A/H1N1, and 54% for B.

Among a pediatric population older than the one to be studied here, Lina, *et al* reported a study of 42 children from 8 to 10 years of age, finding seroconversion rates of 57% (A/H3N2), 76% (A/H1N1), and 29% (B) three weeks after a single 0.5 mL dose of Vaxigrip. [Lina2000] Seroprotection was 83%, 93%, and 100%, respectively. The geometric mean increase was 6.4, 10.1, and 2.3, respectively.

Averaging the 2-dose seroconversion rates in age groups of 6-36 months, similar to those to be studied here (6-23+ months), from the Gonzalez *et al* study and the three studies reported by Saliou *et al*, the mean SC was 85.2% (A/H3N2), 86.4% (A/H1N1), and 56.0% (B).

10.4.2.5 Vaccine Efficacy

Phase III field efficacy trials of influenza vaccine to measure protection against influenza-like symptoms or disease are rare. In one unblinded trial in China among 80 Vaxigrip recipients and 88 observed controls, Jianping *et al* reported reduction of reported symptoms by 84.8% over the 6-month followup period in children 3-6 years of age [Jianping1999]

10.4.2.6 Vaccine Safety Profile

Clinical trials and post-marketing surveillance are the source for knowledge concerning anticipated adverse events (AEs) following administration of Vaxigrip. The overall summary of AEs are quoted here from the product monograph [AventisPasteur2005] :

> "The most frequent side effect of influenza vaccination is soreness at the vaccination site. These local reactions generally are mild and rarely interfere with the person's ability to conduct usual daily activities. (3) [CDC2004] Local redness, swelling, induration and bruising have also been reported. (13) [Data on file at Aventis Pasteur SA]
>
> "Fever, malaise, myalgia, arthralgia, lymphadenopathy, headache, shivering, sweating, fatigue (13) [Data on file at Aventis Pasteur SA] and other systemic symptoms can occur following vaccination with inactivated influenza vaccine and most often affect persons who have had no exposure to the influenza virus antigens in the vaccine (e.g., young children). (3) [CDC2004] (14) [Schiefele1990] These reactions usually disappear within 1 - 2 days without treatment.
>
> "Placebo-controlled trials suggest that among elderly persons and healthy young adults administration of split-virus influenza vaccine is not associated with higher rates of systemic symptoms (e.g., fever, malaise, myalgia and headache) when compared with placebo injections. (3) [CDC2004] (15) [Nichol1996] Prophylactic acetaminophen may decrease the frequency of some side effects in adults. (2) (16) [Aoki1993] "

In the Gonzalez *et al* clinical trial of Vaxigrip among children 6 months to 3 years of age, the only immediate reactions found were 9 cases of *injection site macules or papules* after the 2nd immunization (of 57, 16%), which was deemed allergic in nature. [Gonzalez2000] Delayed local reaction(s) occurred among 9% (5 of 57), including reddened papules of <3cm diameter, and 1 child with severe limb pain preventing voluntary movement. Thirty systemic events within 3 days after an injection were observed in 28% (16) of 57 patients. These comprised 19 events of rhinitis and cough, 5 fevers (38.5° – 38.9° C). Systemic events between 4 days and 30 after vaccination were reported after 30 1st injections and 37 2nd injections, but were deemed common childhood symptoms and not described. One patient with a history of bronchiolitis experienced 2 serious adverse events of bronchospasm occurring 25 days and 2 days after doses 1 and 2, respectively, which were deemed unrelated to Vaxigrip.

In the Saliou *et al* meta-analysis of published and unpublished Vaxigrip studies, safety results from 6 trials (including Gonzalez *et al*) were pooled into a single database. [Saliou2005] Among 337 subjects in the age range of 6 to 36 months, frequencies of *non-severe* AEs were 19% fever, 8.3% pain, 8.3% erythema, 2.7% induration, and 4.2% ecchymosis. *Severe* AE frequencies were 2.1% fever, 0.3% pain, 0% erythema, 0.3% induration, and 0% ecchymosis.

Other very rare AEs reported by the manufacturer [AventisPasteur2005] as a result of post marketing surveillance include:

- Blood and lymphatic system disorders (transient thrombocytopenia, lymphadenopathy)
- Immune system disorders (allergic reactions: pruritis, rash erythematous urticaria, dyspnea, angioneurotic edema, or anaphylactic shock)
- Nervous system disorders (paraesthesia, Guillain-Barre Syndrome [GBS], neuritis, neuralgia, convulsions, encephalomyelitis)
- Vascular disorders (vasculitis, with transient renal involvement in certain cases)

In Canada, a newly-described *oculorespiratory syndrome* (ORS) following influenza vaccination, consisting of early onset (2-24 hours) of bilateral red eyes, and/or cough, wheezing, chest tightness and other respiratory symptoms, was recognized in 2000-2001 and subsequent seasons. [Canada2000, Canada2001a, Canada2001b] The syndrome was generally mild and self-limited. Children less than 9 years of age constituted about 1 percent of cases, which clustered in females (75%) and healthy adults 40-59 years of age. [Boulianne2001] Of the 960 reports satisfying an ORS definition, 96% followed Fluviral® influenza vaccine, manufactured by Biochem Pharma (subsequently Shire Biologicals), which distributed 4 million doses in Canada. [Canada2001b] Only 2% of ORS cases occurred following either the Vaxigrip or Fluzone® products of Aventis Pasteur, which distributed 9 million doses of its two vaccines, manufactured in France and the USA, respectively. A 2001 survey among 642 diabetic children and their siblings who had been vaccinated in a province using only the Fluviral product in the 2000 season identified 83 (13%) who satisfied the case ORS definition, and 1% who required hospitalization. [Skowronski2005]

Studies at multiple laboratories detected an unusually large frequency of aggregates of unsplit virus (up to one-third of total virus) in the Fluviral product, which had recently undergone a change in manufacturing technique. [Skowronski2003] Aggregates were nearly absent in the prior 1999 Fluviral product, as well as in the Fluzone and Vaxigrip products, the latter of which was measured to have a maximum of only 2% unsplit virus. A retrospective cohort study in Québec province suggested the frequency of ORS was equally around 5 percent for recipients of both Fluviral and Vaxigrip products. [DeSerres2003] Since the 2000-2001 season, incidence has declined steadily from 46.6 reported cases per 100,000 doses distributed, to 34.2, 20.6, and 9, in the 2001-, 2002-, and 2003- seasons, respectively. [DeSerres2005]

In a manufacturer-independent side-by-side trial of Vaxigrip and Fluarix® (SmithKline Beecham Biologicals [now GlaxoSmithKline], Rixensart, Belgium) conducted among 18-60 year old adults in 1996-97 in Czechoslovakia, redness at the injection site occurred more frequently (28%) in the Vaxigrip group (n=100) than in the Fluzone one (13%, n=99). [Beran1998] Frequencies of other local reactions

for Vaxigrip vs. Fluarix were 15% / 7%, respectively, for swelling and 50% / 36% for pain (not statistically significant). Systemic reaction rates for Vaxigrip vs. Fluarix were 18% / 11% for headache, 16% / 12% for myalgia, 2% / 1% for shivering, and 13% / 6% for malaise. (Seroconversion and seroprotection rates were generally 10 to 30 percentage points higher for Vaxigrip than for Fluarix, and geometric mean titers were generally fifty percent or more higher, as well. Both vaccines satisfied EMEA requirements for Immunogenicity.[CPMP1997])

10.4.2.7 Guillain-Barré Sequelae of Influenza Vaccination

Guillain-Barré syndrome (GBS) is a neurologic disorder that occurs overall at a rate of about 10-20 cases per million population in the U.S.[Ropper1992] More rarely it may follow influenza vaccination.

Schonberger *et al* described the epidemiology of GBS following the "swine" influenza mass campaign of 1976 in the U.S., which measured a vaccine-attributable risk of GBS of just under 10 cases per million vaccinated. They found the period of increased risk following the swine vaccine strain was concentrated within 5 weeks after vaccination, although lasting for approximately 9 or 10 weeks.

Haber, *et al* found much lower rates for routine seasonal influenza vaccination by analyzing reports to the national Vaccine Adverse Events Reporting System between 1990 and 2003.[Haber2004] The highest frequency was 1.7 cases of GBS per million vaccinees in the 1993-1994 season, and the lowest rate of 0.4 cases per million in 2002-2003. The median interval after vaccination for onset of GBS was 13 days, with about 85% occurring within 4 weeks and about 95% within 8 weeks.

Lasky *et al* identified patients with GBS in four states from hospital discharge data from the 1992-93 and 1993-94 seasons and contacted them and their providers for vaccination history.[Lasky1998] Their adjusted relative risk for GBS among those receiving influenza vaccine was 1.7 times higher (p=0.04) than those who had not been vaccinated. This risk corresponded to slightly more than one additional case of GBS per million persons vaccinated. In 9 of 19 vaccine associated cases where dates were known, the onset of GBS was in the second week after vaccination, all between day 9 and day 12.

At a rate of one per million, the probability of experiencing GBS in this study of 450 children is 0.00045, or about one-twentieth of one percent. This is much less than the risk of serious illness and death in this susceptible age group from influenza disease if they remain unvaccinated, according to ACIP:[CDC2005]

> "Cases of GBS after influenza infection have been reported, but no epidemiologic studies have documented such an association ([ref]209,210). Substantial evidence exists that multiple infectious illnesses, most notably Campylobacter jejuni, and upper respiratory tract infections are associated with GBS (203,211--213).

"Even if GBS were a true side effect of vaccination in the years after 1976, the estimated risk for GBS of approximately 1 additional case/1 million persons vaccinated is substantially less than the risk for severe influenza, which can be prevented by vaccination among all age groups, especially persons aged >65 years and those who have medical indications for influenza vaccination (Table 1) (see Hospitalizations and Deaths from Influenza). The potential benefits of influenza vaccination in preventing serious illness, hospitalization, and death substantially outweigh the possible risks for experiencing vaccine-associated GBS. The average case fatality ratio for GBS is 6% and increases with age (203,214). No evidence indicates that the case fatality ratio for GBS differs among vaccinated persons and those not vaccinated."

10.4.3 Identity of Injection Device (Biojector® 2000, Bioject, Inc.)

The jet injector to be used for delivery of influenza vaccine in the intradermal arm (ID-JI-0.1) of this study is an American-made, hand-held, reusable device powered by standard CO_2 cartridges (see Figure 6). It was introduced into the United States in the mid-1990s and is now cleared for marketing and use in humans in the U.S. (FDA numbers K920631 and K960373),[FDA1997] Canada, the English-speaking countries of the European Union, China, India, Bahrain, Jordan, Korea, Syria, and the United Arab Emirates (2005, personal communication, Susan Frank, Bioject, Inc.). (See: http://www.bioject.com/biojector2000.html)

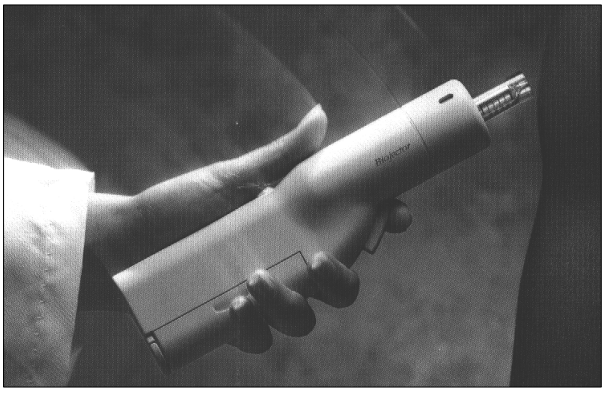


Figure 6. Biojector® 2000 needle-free jet injector.

It uses sterile, disposable cartridges ("syringes"), for which different versions are available for patients of varying size and weight. Subcutaneous or intramuscular injections are achieved by varying the diameter of the orifice in the nozzle of the cartridges (the larger the diameter, the deeper the injection). The no. 2 cartridge (green, 0.10 mm orifice diameter) is for subcutaneous injection for patients of all sizes.[Bioject1997, NIP2005b] Cartridge nos. 3 (brown, 0.15 mm), 4 (blue, 0.20 mm), 5 (silver, 0.25 mm), and 7 (red, 0.36 mm) are for intramuscular injections for infants, toddlers, adults and "large adults", respectively. (An investigational spacer applied to the nozzle of its no. 2 disposable cartridge is to be studied by this protocol, and is described in detail in section 10.4.3.4.)

10.4.3.1 Preclinical and Veterinary Studies in Animals

Various published bench, preclinical, and veterinary studies of the Biojector system have been conducted both prior to and subsequent to initial device licensure for humans. These include studies in mice,[Davis1994, Cartier2000, Singh2001, Trimble2003, Brave2005, Choi2005] rats,[Davis1994] guinea pigs,[Davis1994, Ledwith2000, Manam2000] pigs,[Babiuk2003] rabbits,[Davis1994, Aguiar2001, Hartikka2001] dogs,[Smith1998] rhesus macaque monkeys,[Barouch1998, Barouch2000a, Barouch2000b, Rogers2001, Amara2001, Amara2002, O'Neill2002, Rogers2002, Raviprakash2003, Sadagopal2005] cynomolgus macaque monkeys,[Sullivan2000, Rao2005] aotus monkeys,[Gramzinski1998] and chimpanzees.[Davis1996]

Many of the postlicensure studies using the Biojector involved the delivery of investigational DNA vaccines.[Mumper2003, Trimble2003] See section 10.4.3.5 for additional details on some of these animal studies that included the intradermal spacer. Analysis by two high performance liquid chromatography methods of 18 different proteins ejected by a similar investigational jet injector (Iject™, also from the Bioject company) that shares a similar performance profile to the Biojector found no damage to the proteins nor the formation of aggregates.[Benedek2005]

As-yet unpublished animal research using the Biojector includes the following, among other collaborations between Bioject, Inc. and the U.S. FDA, the NIH, Imclone Systems, Emory University, Georgetown University, Johns Hopkins, and the Universities of Kentucky, Massachusetts, Michigan, and Puerto Rico (source: Bioject, Inc., 2005):

- Antex Biologicals, Inc. (now Bioport): DNA vaccines for chronic bacterial infection
- Auburn University: DNA vaccines for canine/feline parvovirus
- Corixa, Inc. and Oregon Regional Primate Research Center: adjuvanted DNA vaccines in rhesus macaques is enhanced by Biojector delivery or microsphere encapsulation (tuberculosis antigen IM and ID) [Evans2001, Evans2002, Mossman2003]
- Genencor International: experimental animal models for hepatitis A and C, and human papillomavirus vaccines
- Genetronics, Inc., and Veterinary Infectious Disease Organization, University of Saskatchewan: DNA vaccination in pigs with DNA plasmid for bovine herpes virus[Baizer2001, Babiuk2003]
- Genzyme, Inc.: Intradermal administration into guinea pigs of recombinant adenovirus as cancer vaccine model[Perricone2000]
- Iowa State University: DNA vaccines for swine
- Memorial Sloan Kettering Cancer Center: DNA melanoma vaccine study in dogs (http://www.mskcc.org/mskcc/html/13199.cfm)
- Universities of Toronto and Wisconsin: multiantigen HIV vaccines in primates

10.4.3.2 Clinical Studies of Licensed Drugs and Vaccines

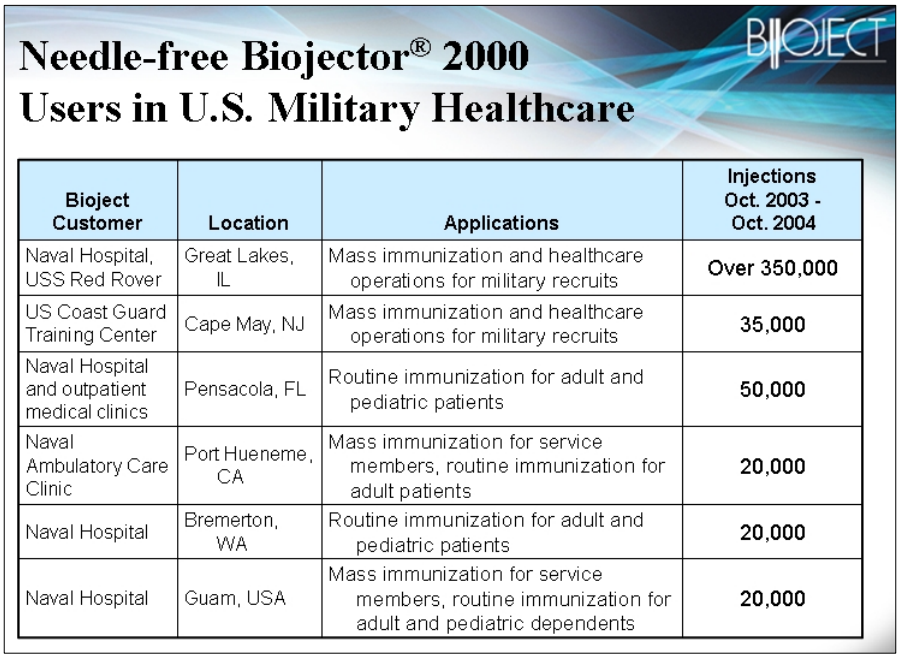
Human trials using the Biojector and its standard intramuscular or subcutaneous cartridges have demonstrated satisfactory immune responses and safety for vaccination

with licensed vaccines against influenza,[Jackson2001] yellow fever,[Jackson1993] and hepatitis A.[Williams2000] In one study, alum-adjuvanted hepatitis B vaccine produced about twice the frequency of local reactions by Biojector IM than control needle-syringe IM, but comparable seroconversion rates.[Matheï1997] In a study of apprehension among 6th grade children receiving hepatitis B by Biojector IM on one dose and needle-syringe on the other, pre- and post-injection anxiety scores were similar; however 61% of subjects preferred the Biojector (p=0.08).[Polillio1997] Other studies have described effective Biojector use in administering anesthesia, both local[Gerbert1996, Florentine1997, Koenig2004] and general,[Greenberg1995, Baer1996, Bennett1998, Phero1998, Fine2004] as well as for heparin therapy.[Baer1996]

10.4.3.3 Clinical Studies of Investigational Drugs and Vaccines

Published reports have documented use of the Biojector system in administering investigational drugs and vaccines such as (1) carcinoembryonic antigen in recombinant viral vectors to cancer patients,[Marshall1999, Marshall2000, Conry1999] (2) malaria vaccines,[Wang2001, Epstein2002] and (3) HIV/AIDS[Boyer2000] (see section 10.4.3.6 for additional details on some of these and other studies that included the investigational ID spacer).

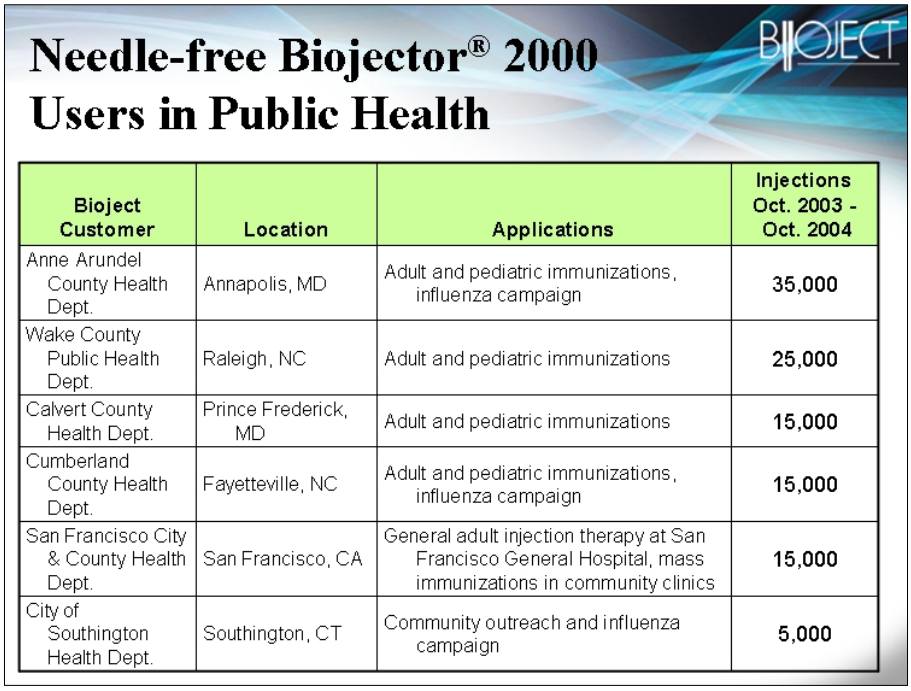
As described in the first two paragraphs of section 9.3.3, the Biojector system is in use by a number of immunization providers since its introduction in the mid-1990s. Bioject, Inc. reported that about one million injections per year are performed with the device, as estimated from cartridge sales (personal communication, Kurt Lynam, 2000). The U.S. Navy uses the regular system for routine use among adult and pediatric dependents of sailors, and also speeds up mass vaccination of naval recruits by attaching large-volume nitrogen tanks to avoid replacing empty CO_2 cartridges every 10 to 15 injections (see Figure 7). The system is also used in a number of public health (see Figure 8) and pediatric settings (see Figure 9) around the United States.



Needle-free Biojector® 2000 Users in U.S. Military Healthcare

Bioject Customer	Location	Applications	Injections Oct. 2003 - Oct. 2004
Naval Hospital, USS Red Rover	Great Lakes, IL	Mass immunization and healthcare operations for military recruits	Over 350,000
US Coast Guard Training Center	Cape May, NJ	Mass immunization and healthcare operations for military recruits	35,000
Naval Hospital and outpatient medical clinics	Pensacola, FL	Routine immunization for adult and pediatric patients	50,000
Naval Ambulatory Care Clinic	Port Hueneme, CA	Mass immunization for service members, routine immunization for adult patients	20,000
Naval Hospital	Bremerton, WA	Routine immunization for adult and pediatric patients	20,000
Naval Hospital	Guam, USA	Mass immunization for service members, routine immunization for adult and pediatric dependents	20,000

Figure 7. Use of Biojector system in U.S. military, 2003-2004. Source: Bioject, Inc.



Needle-free Biojector® 2000 Users in Public Health

Bioject Customer	Location	Applications	Injections Oct. 2003 - Oct. 2004
Anne Arundel County Health Dept.	Annapolis, MD	Adult and pediatric immunizations, influenza campaign	35,000
Wake County Public Health Dept.	Raleigh, NC	Adult and pediatric immunizations	25,000
Calvert County Health Dept.	Prince Frederick, MD	Adult and pediatric immunizations	15,000
Cumberland County Health Dept.	Fayetteville, NC	Adult and pediatric immunizations, influenza campaign	15,000
San Francisco City & County Health Dept.	San Francisco, CA	General adult injection therapy at San Francisco General Hospital, mass immunizations in community clinics	15,000
City of Southington Health Dept.	Southington, CT	Community outreach and influenza campaign	5,000

Figure 8. Use of Biojector system in public health, 2003-2004. Source: Bioject, Inc.

Needle-free Biojector® 2000 Users in Pediatrics

Bioject Customer	Location	Applications	Injections Oct. 2003 - Oct. 2004
Cobb County Public Health Dept.	Marietta, GA	Pediatric immunizations, influenza campaign	30,000
Brownsville Community Health Center	Brownsville, TX	Pediatric immunizations, influenza program	20,000
Children's Hospital of Oakland	Oakland, CA	Pediatric immunizations, influenza program	12,000
Children's Hospital of Miami	Miami, FL	Pediatric immunizations, community outreach program	12,000
Torrance Pediatrics	Torrance, CA	Pediatric immunizations, private practice	5,000
Gila River Healthcare Corp.	Sacaton, AZ	School-based immunization program, Pima Indian Reservation	2,500

Figure 9. Use of Biojector system in general pediatrics, 2003-2004.
Source: Bioject, Inc.

10.4.3.4 Investigational Spacer

This investigational spacer to be used to administer intradermal injections is a high density polypropylene cylindrical tube that attaches by friction fit to the front of the Biojector no. 2 (subcutaneous) cartridge (see Figure 10).

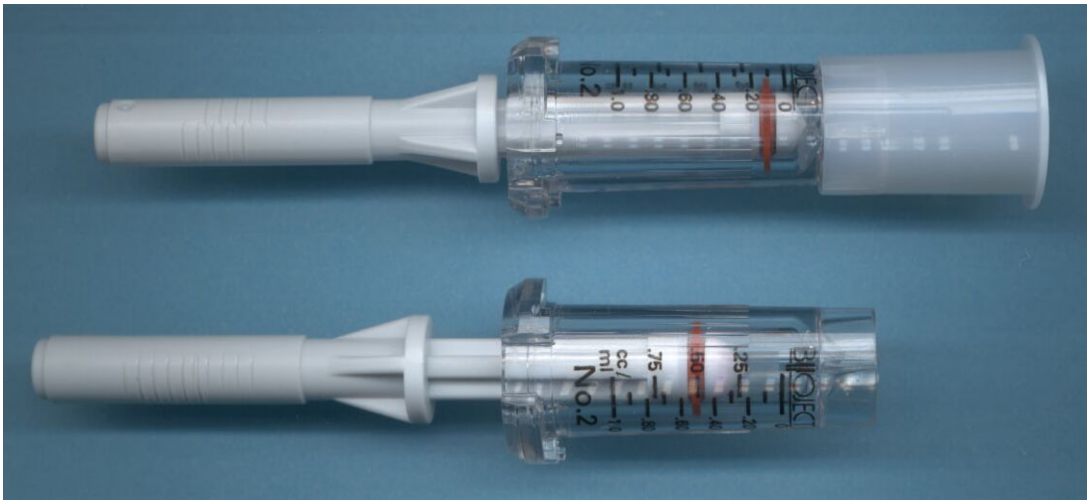


Figure 10. Investigational intradermal spacer (top) fitted to no. 2 Biojector cartridge (by itself, bottom).

The spacer creates a gap of 2 cm between the skin and the orifice in the nozzle of the cartridge, weakening the stream enough to achieve the desired intradermal delivery (see Figure 11). This variation of jet injection practice has been demonstrated by others since at least the 1970s and was discussed in detail earlier in sections 9.3.1 and 9.6.3.

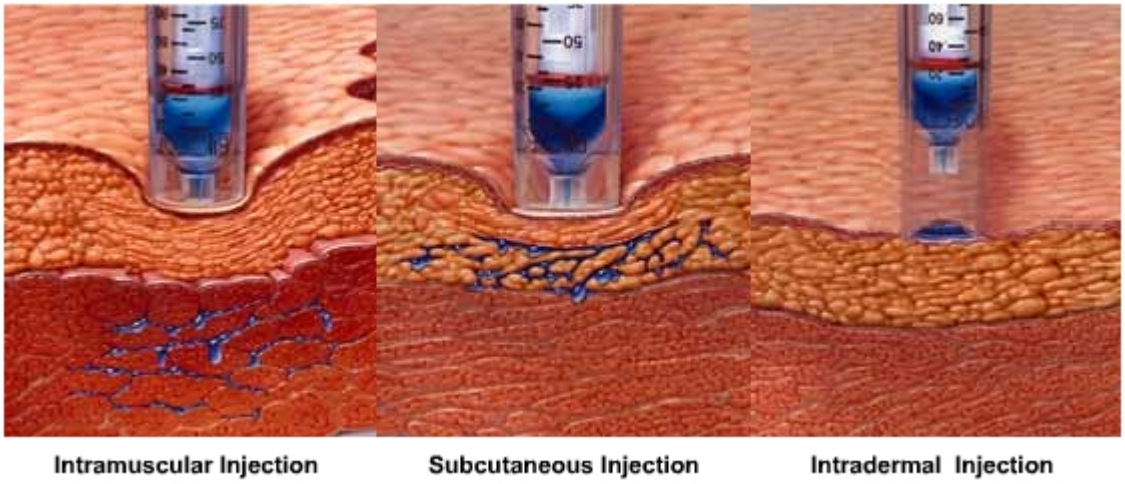


Figure 11. Artist's rendition of dose distribution for Biojector needle-free jet injections in human tissues. Intradermal cartridge on right reflects manufacturer's intention to incorporate a spacer into the nozzle of a future product, rather than as a separate attachment which now exists. Source: Bioject, Inc.

10.4.3.5 Preclinical Studies of the Biojector ID Spacer

A number of animal studies with the Biojector spacer have been performed to demonstrate safety and/or efficacy of the investigational spacer used to administer intradermal injections with the Biojector cartridge and injector.

10.4.3.5.1 Mice, University of Kentucky

BALB/C mice were immunized by subcutaneous (SC) needle-syringe (N-S) injection or intradermally (ID) by the Biojector system and spacer with three doses of nanoparticles coated with plasmid DNA (pDNA) expressing β-galactosidase, or the pDNA alone, or the protein alone with alum.[Cui2003] For pDNA alone, there was no difference in IgG titers to the antigen between the two routes of administration. But for the particle pDNA, ID jet injection generated 20-fold higher antibody titers than SC by N-S.

10.4.3.5.2 Mice, Rotavirus Antigen, Cincinnati Children's Hospital

The rotavirus vaccine antigen VP6 was administered by various routes to mice at the Cincinnati Children's Hospital to explore non-oral routes of vaccination to avoid gastrointestinal side effects of the vaccine.[Choi2005]

Groups of the BALB/c subjects were given the antigen, with or without a modified heat-labile *E. coli* toxin as an adjuvant, by (1) skin patch, (2) Biojector intradermal injection followed by skin patch with adjuvant only over the injection site, or (3) intranasal instillation.

Only the intranasal route produced significant reductions in fecal shedding following oral rotavirus challenge. The Biojector ID route, however, with or without adjuvant, produced from 35 to 100 times as much specific IgG immunoglobulin as did the comparable patch vaccination, and also exceeded the antibodies generated by the intranasal route. The authors speculated that the inability of the Biojector ID method to induce protection was because the strongly hydrophobic VP6 antigen was not effectively reaching the epidermis. Needle-syringe ID and SC delivery did not have this problem, although only the SC route produced protection from shedding.

10.4.3.5.3 Pigs, Hepatitis B Vaccine, University of Saskatchewan

Researchers at the Vaccine and Infectious Disease Organization and collaborators at the University of Saskatchewan studied vaccination with a DNA plasmid encoding for hepatitis B surface antigen (HBsAg) and a commercial human recombinant hepatitis B vaccine (GlaxoSmithKline).[Babiuk2003] The DNA plasmid antigen was administered intradermally on the abdomen of 4-to-6-week old outbred pigs, using either Biojector with spacer or a conventional ID needle injection, both with and without followup electroporation of the injection site (60 ms electrical pulses of 60-80 volts, believed to permeabilize cell membranes and improve uptake). Two additional groups of pigs received conventional liquid vaccine containing hepatitis B surface antigen (HBsAg) by either ID Biojector (2 injections totalling 0.5 mL per animal) or IM needle injection (0.5 mL). All six groups were boosted at week 8 with the conventional vaccine by IM needle, except the group which had been primed with this same antigen by ID Biojector received the booster by the same method and route as before.

By the time of the 8-week booster, most of the conventional vaccine recipients (4/5 for Biojector ID, 5/5 for needle ID) had developed antibody responses ≥10 IU and OD ≥2 standard deviations over background for both the ELISA and AUSAB® assays. On the other hand, only the Biojector DNA plasmid group with (2/5) or without (1/5) electroporation achieved a response on both assay, while none of the ID needle groups did so. Two weeks after the booster, all electroporation DNA plasmid groups (Biojector ID and needle ID), as well as the groups receiving only the conventional vaccine achieved 100% immune response (5/5 or 4/4) on both assays. The DNA plasmid groups without electroporation -- both Biojector ID and needle ID -- had 60% (3/5) responses to the ELISA assay, while the former's response was 80% (4/5) and the latter's 100% (5/5) to the AUSAB assay.

The authors did not comment on observable local abdominal reactions around the injection sites. Histological examination macrophage infiltration in the dermis and epidermis attributable to the electroporation voltage.

10.4.3.5.4 Mice, guinea pigs, monkeys, HIV Vaccine, Emory University and CDC

In pre-primate preparatory studies at the Yerkes National Primate Research Center, Emory University, Balb/c mice and Hartley guinea pigs were injected with modified vaccinia Ankara expressing HIV (MVA/HIV) antigens by either Biojector intradermally (ID) or needle-syringe intramuscularly (IM). Wyatt2004 Both routes were found to be equivalent for raising neutralizing antibody. The authors did not note any difficulties with intradermal jet injection in the experimental animals.

Twenty-four rhesus macaque monkeys at CDC's Lawrenceville, GA animal facility received two priming doses of DNA vaccine by Biojector 2000 by either the ID (with spacer) or IM routes (without spacer). Amara2001, Amara2002, Buge2003 They were then boosted with a single MVA/HIV dose by needle-syringe ID or IM. The ID delivery of DNA priming was about 10 times more effective than IM for generating *gag* antibody. At comparable dose levels, the ID groups achieved higher ELISPOT assay results and proliferative responses than the IM groups. Amara2001

Following intrarectal challenge of pathogenic virus administered 7 months after vaccination, animals in both routes of delivery showed effective control of virus. Gp120 protein administered in some animals alongside the second prime and the boost doses 2 and 3 did not improve protection. Buge2003 Two hundred weeks post-challenge, 22 of 23 animals had successfully controlled the viremia of their challenge strain, demonstrating long-term immune control as a result of vaccination. Sadagopal2005

10.4.3.5.5 Rhesus Macaques, DNA HIV Vaccine, University of Puerto Rico

At the Caribbean Primate Research Center in San Juan, six groups of five rhesus macaque monkeys each were vaccinated three times at 13-week intervals with a 2 mL solution of DNA expressing various HIV antigens with or without various cytokine genes as adjuvant.O'Neill2002 Except for a seventh naïve control group, on each vaccination day each animal received two IM injections of 0.5 mL each in the triceps muscle by Biojector, plus 10 ID injections of 0.1 mL each on the thigh by Biojector with spacer.

At 39 weeks after the first priming dose, all animals except the naïve controls were boosted with a virus-like particle expressing simian immunodeficiency virus (SIV) antigens, in the presence (groups 1, 3, 5) or absence (groups 2, 4,

6) of recombinant IL-12 cytokine as adjuvant. At 47 weeks, all animals were challenged intrarectally with the highly pathogenic SIVsmE660 virus strain.

All but one monkey became infected by the challenge virus, as evidenced by plasma viral RNA. Compared to animals receiving the antigens only, however, monkeys administered as well the cytokine adjuvants showed complete protection from clinical disease for at least 14 months (including the uninfected subject). Antigen-only groups evidenced clinical illness and lower survival. Vaccinated animals demonstrated significantly lower peak plasma viral loads than the controls. The authors did not comment on local reactions to the Biojector IM and ID injections, if any.

10.4.3.5.6 Infant Rhesus Macaques, DNA Measles Vaccine, University of Maryland

Preclinical studies in infant rhesus macaque monkeys were conducted at the Center for Vaccine Development of the University of Maryland, Baltimore, as preparation towards planned human studies of an experimental DNA-construct measles vaccine that might ultimately protect human infants as young as 3 months of age (Karen Kotloff, personal communication, 2005). Current measles vaccines are not generally used in children younger than 9 months of age because of interference from transplacental maternal antibody which lowers efficacy.

The vaccination regimen was administered at the Johns Hopkins University Rhesus colony to six monkeys less than 2 months of age as a model for young human infants. Under suitable anesthesia of both mother and infant, each infant was vaccinated with the DNA measles vaccine in two intradermal doses of 0.1 mL each at different body sites on both days 0 and 28, using the Biojector system and intradermal spacer (see Figure 12 and Figure13). All were boosted at around day 56 with a conventional, live attenuated, measles vaccine administered in an aerosol via pulmonary inhalation.

Figure 12. Intradermal injection of infant rhesus monkey using Biojector spacer. Source: Center for Vaccine Development, Univ. of Md.

Regarding safety, the researchers noted that "all vaccine doses were well tolerated". "No adverse events or complications were observed and the young animals showed a steady increase in weight throughout the experiment." (Normal weight gain in this animal model is a good indicator for the absence of illness, discomfort, or complications.)

Figure13. Desired visible wheal resulting from Biojector intradermal injection in rhesus macaque monkey. Source: Center for Vaccine Development, Univ. of Md.

Regarding immune response, all monkeys developed good to excellent plaque reduction neutralization antibody titers following either the first or second intradermal vaccinations, which were further strengthened after the aerosol booster. Upon challenge on day 220 with wild type measles virus, all subjects were protected from viremia and symptomatic illness, except for signs of rash in one and a few spots in another.

10.4.3.5.7 Rabbits, Monkeys, Malaria Vaccine, Naval Medical Research Center

As preparatory work in advance of planned human trials of a DNA plasmid *falciparum* malaria circumsporozoite vaccine (see section 10.4.3.6.2), Navy researchers compared administration on day 0, weeks 4 and 8, and month 7 by three routes to groups of albino rabbits.[Aguiar 2001] These routes were (1) 0.5 mL (500 μg) vaccinated IM by needle-syringe (N-S), (2) 0.5 mL IM by standard no. 2 Biojector needle-free cartridge, (3) 0.35 mL IM by standard no. 2 Biojector needle-free cartridge plus three simultaneous doses of 0.05 mL ID by Biojector cartridge with intradermal spacer, and (4) unvaccinated controls. All groups 1 to 4 also received at month 11 a boost by IM needle-syringe with ALVAC canarypox vaccine expressing the same protein as the DNA prime.

After four doses, the IM Biojector group developed indirect fluorescence antibody test (IFAT) geometric mean titers (GMTs) to sporozoite antigen that were 3-fold higher than GMTs of the N-S group, and GMTs of the IM/ID Biojector group were 8-fold higher. After the canarypox boost, GMTs were 18-fold and 16-fold higher in the Biojector groups, respectively, than in the N-S group. The researchers did not report any monitoring of local adverse effects of the injections.

The Navy also studied rhesus macaque monkeys undergoing malaria vaccination with the Biojector used for both IM and ID injections.[Rogers2001, Rogers2002] Three groups of four monkeys each received three doses of a mixture of four DNA plasmids encoding multiple *Plasmodium knowlesi* antigens, followed by recombinant canarypox boosting. The groups comprised (1) IM via N-S, (2) IM via Biojector, and mixed IM and ID injections, all with the Biojector. In addition, there were tour unimmunized controls. All immunization routes induced antibodies against multiple antigens. Following *P. knowlesi* challenge, all controls were infected with overwhelming parasitemia requiring life-saving treatment, while 2 of 11 immunized monkeys achieved “sterile” protection, and the remaining 7 resolved their parasitemias spontaneously.

10.4.3.5.8 Mice, HIV Vaccine, Karolinska Institute, Sweden

A DNA vaccine encoding nine HIV protein antigens was administered IM or ID with novel adjuvant to BALB/c mice by Biojector system, inducing strong HIV *env* and *gag* specific cellular and humoral immune responses.[Brave2005] A subsequent GMP-produced lot of this vaccine intended for human use was again administered to mice ID by Biojector and IM with GM-CSF adjuvant by needle-syringe, with control animals receiving placebo by both routes. Splenocyte interferon γ was produced in both group, although higher in the adjuvanted IM group. No mice showed toxic effects on health status, hematology, clinical chemistry, or histopathology.

The authors concluded that this vaccine delivered intradermally by jet injector was safe and efficacious in inducing high levels of specific $CD8^+$ T cells and antibodies. The vaccine was subsequently approved by the Swedish Medicinal Products Agency for a clinical phase I trial, which began vaccination in February 2005 (see section 10.4.3.6.6).

10.4.3.6 Clinical Studies of the Biojector ID Spacer

A number of completed and ongoing human trials using the investigational Biojector spacer have been conducted and found its use safe and tolerable (see Figure 15 and Figure 14). These studies were approved for human subjects by the national regulatory authorities of the United States (FDA), the United Kingdom (Medicines Control Agency), Sweden (personal communication, Richard Stout, Biojector, Inc., 2006), Finland (National Agency for Medicines), and South Africa (Medicines Control Council). Copies of confidential documentation from these agencies are available via the manufacturer upon request by ethical review committees.

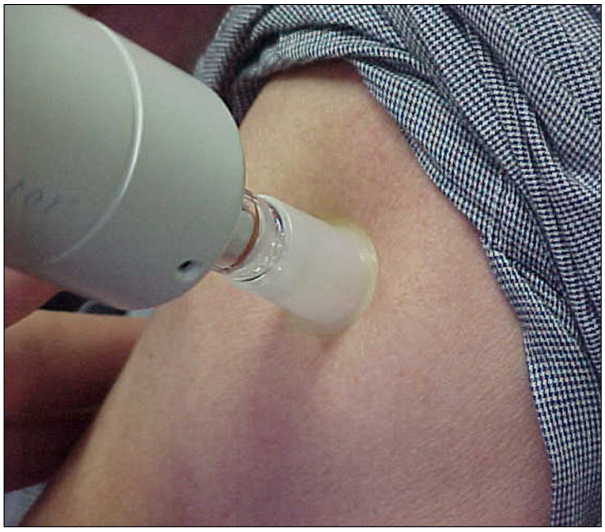


Figure 15. Biojector® 2000 injector with investigational spacer on cartridge nozzle administering intradermal injection.

Figure 14. Intradermal wheal visible on skin after use of Biojector® 2000 intradermal spacer.

10.4.3.6.1 Local anesthesia in adults, University of Illinois

Anesthesiologists at the University of Illinois Medical Center in Chicago used intradermal spacers on two different jet injectors, including the Biojector 2000, to anesthetize with lidocaine the dorsum skin of the hand of 25 consenting adult patients each prior to usually painful intravenous catheterization.[Zsigmond1999a, Zsigmond1999b] Another 25 patients received control anesthesia administered intradermally by 25-gauge needle-syringe. Patients applying visual analog scales (VAS) for pain reported zero pain during the lidocaine injections as well as subsequent cannulations in much higher proportions for both jet injectors (≥84%) compared with the control needle-syringe injections (≤24%).

10.4.3.6.2 Malaria DNA vaccine in adults, U.S. Navy

Among 21 human volunteers, investigators at the U.S. Naval Medical Research Center carefully described the safety and tolerability of a malaria DNA vaccine delivered either IM by needle-syringe, IM alone by Biojector 2000, and both IM and ID by Biojector 2000 using the investigational spacer.[Wang2001, Epstein2002] In this trial, five subjects in the third study arm received three "identical" ID injections by Biojector in one arm (with ~1 cm between each bleb), and a standard IM injection by Biojector in the other arm at each of three visits. Because of their proximity, the blebs were treated as a single immunization site and the IM site as a separate immunization site in the assessment of local reactogenicity.

The investigators found that:

- Subjects receiving the Biojector injection (IM or ID) preferred this mode of administration relative to their recall of previous needle injected vaccinations when questioned at each immunization time point (26 preferring Bioject versus 3 preferring needle injection).
- At 6 months, all volunteers indicated that they would choose Biojector over needle and syringe for future immunization. Reported reasons for preferring the Biojector included ease of administration, sterility of single-use applicators, and less pain associated with injection. However, this was a nonblinded, nonplacebo-controlled comparison and there was no comparison group that received an ID injection by needle and syringe.
- Of the 172 AEs reported by Biojector recipients that were thought to be definitely or probably related to immunization, all were localized to the injection site except for 3 mild constitutional symptoms.
- Of these 172 AEs, 169 were mild and 3 were moderate (all 3 moderate AEs were in the IM group). Two of the mild reactions following ID injection lasted 10 days or more (bruising).

Regarding immune response to the DNA vaccine, none of the vaccinees, even from the IM needle-syringe control study arm, developed specific antibodies to the malaria antigens, despite positive results from prior animal studies in rabbits and monkeys (see section 10.4.3.5.7).

10.4.3.6.3 Influenza in the Frail Elderly, Eastern VA Medical Center

Biedenbender and colleagues implemented an elegant double-blind study design in comparing the 2001-2002 season influenza vaccine administered IM by N-S (0.5 mL, n = 44) versus ID by Biojector (0.1 mL, n = 45) among randomized frail nursing home patients who received a simultaneous injection of placebo by the other method and volume into the opposing arm.[Biedenbender2002] This design permitted each subject to compare the perceived pain between the two injections using visual analog and verbal pain scales.

The mean age of the subjects receiving vaccine IM by N-S was 83 years, and 81 years for those vaccinated ID by Biojector (minimum inclusion criteria age was 60 years). Immune responses were significantly poorer in the ID-vaccinated group, whose hemagglutination inhibition GMTs on day 28 were 50.7, 14.1, and 23.3 for A/Panama, A/ New Caledonia, and B/Victoria, respectively. These were roughly half the GMTs of 100.5, 28,8, and 52.4, respectively among the IM-vaccinated group. Of the IM-vaccinated group, 42 of 44 (95%) demonstrated either a 4-fold rise in titer or an HI titer of $\geq$40 to all three virus antigens, compared to 31 of 45 (69%) of the ID-vaccinated group.

Local reaction frequency rates were not reported. But redness at the vaccine injection site was noted to be significantly more frequent on days 1 ($p=0.035$) and 2 ($p=0.009$) among those vaccinated ID than IM, while similar redness frequencies were reported for the ID and IM placebo injections. The other two local reactions monitored – arm swelling and regional adenopathy – were similar between the two vaccination routes. The pain scores reported immediately after vaccination was similar between the ID and IM vaccination groups. But when each patient compared the immediate (day 0) pain between his or her two injections, ID injections had less pain than IM ones on both the visual ($p<0.001$) and verbal ($p=0.02$) pain scales, but there was no intrapatient difference by days 1 and 2. The researchers indicated no skin tears or other local trauma from the vaccinations.

Systemic symptoms such as headache, lethargy, nausea, vomiting, malaise, fever, and rash were reported in fewer than 7 percent of each study group, and none were significantly more common in one than the other. Outcomes in the 60 day followup period included myocardial infarction, leg gangrene, hypotension, respiratory distress, rectal bleeding, and death, however these

were deemed by the investigators as unrelated to the study and not associated statistically with either study arm.

10.4.3.6.4 Plasmid DNA Lymphoma Vaccine, Stanford University

Excised neoplastic peripheral lymph node(s) of 12 adult patients with B-cell lymphomas were used to create patient-specific DNA plasmids expressing each patient's tumor-specific variable region of a chimeric immunoglobulin molecule.[Timmerman2002] These were administered as vaccines to the patients after completion of chemotherapy in three series of vaccinations, each with three monthly doses. The first series used IM needle injections in escalating dose amounts of antigen. Beginning 17 months later for the second series, and 14 months thereafter for the third series, the highest dose in series 1 (1800 µg) was administered by Biojector system, of which 80% was by IM cartridge (0.8 mL in one posterior upper arm) and 20% in two adjacent ID-spacer injections of 0.1 mL in the opposite arm. In series 3, GM-CSF plasmids were also injected. The researchers carefully observed local and systemic reactions for one hour, and patients used a diary card to record subsequent reactions.

After vaccination series 2, 9 of 12 patients demonstrated immune responses, either humoral (6), T cell (6) or both (3). After series 3, 7 of the 9 responders to series 2 continued to display the measured immune responses, and there was one new responder.

The incidence and severity of both the series 2 Biojector IM injections were indistinguishable from that experienced after the needle-syringe IM injections of series 1. Similarly, the series 3 injections were indistinguishable from the series 2 for local reactions. The authors wrote, "No appreciable local reactions were observed at the sites of ID inoculation" and "No systemic toxicity was noted".

10.4.3.6.5 Colorectal Cancer Vaccine, Oxford Biomedica, UK

In a trial among patients with colorectal cancer (Duke's stage D), the Biojector system with intradermal spacer was used for six patients receiving three doses ($1x10^8$ pfu in 0.1 mL at 0, 4, and 8 weeks) of a modified vaccinia Ankara (MVA) vector expressing tumor-associated antigen 5T4 (TroVax®), and compared with 16 patients receiving the same antigen intramuscularly by needle-syringe in dose escalation ($5x10^7$ to $5x10^8$ pfu).[Harrop2003, HarropMs, Reinis2004] If clinical or immunological response was detected, two more doses at 14 and 20 weeks were offered.

Most patients showed proliferative responses to the protein or peptide 5T4 antigen, although responses tended to be greater and less transient in the IM than ID groups. The most commonly reported treatment-related adverse events were non serious, local ones relating to the injection site. There was a

notably higher incidence of injection site erythema in the intradermal injection group (3 of 6, 50%).

10.4.3.6.6 Adult Volunteers, HIV vaccine, Karolinska Institute

At the Karolinska Institute in Stockholm, Sweden in February, 2005, the first human volunteers began receiving vaccination in an ongoing HIV vaccine trial using a multigene DNA vaccine (animal studies described earlier in section 10.4.3.5.8).[Brave2005]

10.4.4 Method of Assigning Participants to Treatment Groups

For each phase of the study (I and II), an independent, outside party (e.g., a member of the Data and Safety Monitoring Board - DSMB) will determine in advance the injection method to be used for each participant identification (ID) number to be assigned in the study:

- IM-NS-0.25 (control) (intramuscular, needle-syringe, full dose)
- IM-NS-0.1 (intramuscular, needle-syringe, reduced dose)
- ID-JI-0.1 (intradermal, jet injector, reduced dose)

The outside party shall provide copies of these treatment group assignments for each study ID number to at least two [other] members of the DSMB. These records shall remain confidentially in their custody in separate locations outside of the Dominican Republic and released only at the time and in the manner as described in this protocol.

A study nurse will assign permanent participant ID numbers consecutively to each patient in the chronological order in which the corresponding informed consent form is signed and he or she is enrolled.

10.4.4.1 Equal Allocation by Sextuplet Blocks

Treatment group assignments will be made by generating a series of "sextuplet" blocks of 6. For each block, the independent party will assign in random order two injections from each of the three study arms to a randomly selected position (1st, 2nd, 3rd, 4th, 5th, or 6th) within the block. Thus, there will be 36 (3! x 3!) possible orders for any given block of six, as illustrated in Table 4.

Table 4. Examples of random sextuplet blocks for allocating samples

		Sequence Position					
		1st	**2nd**	**3rd**	**4th**	**5th**	**6th**
Sample Blocks	**A.**	IM-NS-0.25	IM-NS-0.25	IM-NS-0.1	IM-NS-0.1	ID-JI-0.1	ID-JI-0.1
	B.	IM-NS-0.25	IM-NS-0.1	IM-NS-0.25	IM-NS-0.1	ID-JI-0.1	ID-JI-0.1
	C.	ID-JI-0.1	IM-NS-0.1	IM-NS-0.1	ID-JI-0.1	IM-NS-0.25	IM-NS-0.25
	D.	IM-NS-0.1	IM-NS-0.25	IM-NS-0.25	ID-JI-0.1	ID-JI-0.1	IM-NS-0.1
	etc.						

For combined phases I and II, a total of 80 blocks will be prepared (480 assignments), 30 more than the target sample size (n=450), in case of dropouts or other contingency. This system will provide for equal or nearly equal sizes of each study group throughout the enrollment process.

10.4.4.2 Avoiding Block Counting

To prevent "block counting" to predict the route of vaccination for a child, the first block will be split randomly into two parts (1+5, 2+4, 3+3, 4+2, or 5+1), with its first segment applicable to the initial participant ID numbers, and its remainder applied to the final ID numbers.

For example, if block 1 were randomly determined to follow sample sequence A in Table 4 above, and is then randomly split into "1 + 5", then participants would be assigned their injection route as follows:

Last 3 digits are Participant ID no.* ↓	Vaccination Group
CDC-ISO-4785-**001**	IM-NS-0.25 (as per block 1, example A)
CDC-ISO-4785-**002** – **475**	(in accordance with blocks 2 through 79)
CDC-ISO-4785-**476**	IM-NS-0.25 (as per block 1, example A)
CDC-ISO-4785-**477**	IM-NS-0.1 (as per block 1, example A)
CDC-ISO-4785-**478**	IM-NS-0.1 (as per block 1, example A)
CDC-ISO-4785-**479**	ID-JI-0.1 (as per block 1, example A)
CDC-ISO-4785-**480**	ID-JI-0.1 (as per block 1, example A)

Similarly, if block 1 were randomized to follow sequence D in Table 4 above, and is randomly split 4+2, then participants would be vaccinated as follows:

Participant ID no.* ↓	Vaccination Group
CDC-ISO-4785-**001**	IM-NS-0.1 (as per block 1, example D)
CDC-ISO-4785-**002**	IM-NS-0.25 (as per block 1, example D)
CDC-ISO-4785-**003**	IM-NS-0.25 (as per block 1, example D)
CDC-ISO-4785-**004**	ID-JI-0.1 (as per block 1, example D)
CDC-ISO-4785-**005** – **478**	(in accordance with blocks 2 through 79)
CDC-ISO-4785-**479**	ID-JI-0.1 (as per block 1, example D)
CDC-ISO-4785-**480**	IM-NS-0.1 (as per block 1, example D)

10.4.4.3 Allocation Group Randomization Cards

The study investigators shall provide the outside, independent party with sufficient numbers of *allocation group randomization cards*, samples of which are pictured in **Figure 16** through **Figure 19** below.

The text of the mock injection card to avoid the extravaccination of a 3rd "insurance" dose on day 56 (**Figure 19**, section) translates as follows in English:

Do NOT administer vaccine to this child.

Apply a bandage even though there is no injection

- Keep the child in the injection room por the time it usually takes to give an injection.
- Do not inform the parents or doctors that no injection was given.
- Revealing this "mock injection" can compromised the blinding of the study.
- Destroy this card before inviting the parents to enter or yourself leaving the room.

Apply a bandage in the place where an injection would be given: in the left thigh for children <12 months of age. In the left deltoid for children 12 months and older.

Figure 16. Randomization card for "IM-NS-0.25" group.

Administre a este niño/a 0.25 ml de vacuna
por vía intramuscular con aguja y jeringa.
Verifique antes de administrar que la dosis y el método emparejen esta tarjeta.
Destruya esta tarjeta antes de invitar a padres en sitio o de salir.
Muslo izquierdo hasta 12 meses.
Llene por favor la jeringa hasta que el sello superior negro del émbolo está por debajo de la marca índice que indica 0.25 mililitro.
0.1
0.2
0.4
0.5
Deltoideo izquierdo más de 12 meses.
Ensayo Clínico de Inmunización con Vacuna Influenza (INF, gripe)
Hospital Infantil Dr. Robert Reid Cabral

Figure 17. Randomization card for "IM-NS-0.1" group.

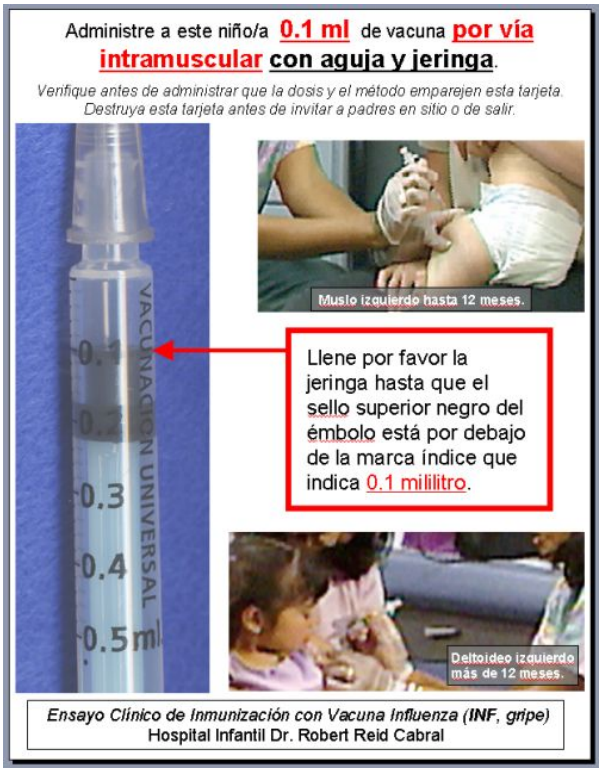
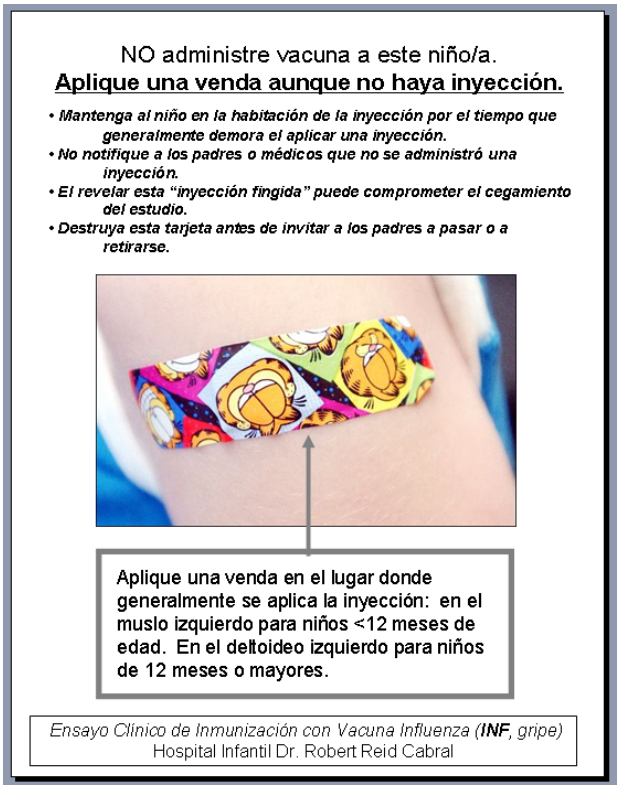

Administre a este niño/a 0.1 ml de vacuna por vía intramuscular con aguja y jeringa.
Verifique antes de administrar que la dosis y el método emparejen esta tarjeta.
Destruya esta tarjeta antes de invitar a padres en sitio o de salir.
VACUNACION UNIVERSAL
0.3
0.4
0.5m
Muslo izquierdo hasta 12 meses.
Llene por favor la jeringa hasta que el sello superior negro del émbolo está por debajo de la marca índice que indica 0.1 mililitro.
Deltoideo izquierdo más de 12 meses.
Ensayo Clínico de Inmunización con Vacuna Influenza (INF, gripe)
Hospital Infantil Dr. Robert Reid Cabral

Figure 18. Randomization card for "ID-JI-0.1" group.

Administre a este niño/a 0.1 ml de vacuna
por vía intradérmica con inyector a chorro.
Verifique antes de administrar que la dosis y el método emparejen esta tarjeta.
Destruya esta tarjeta antes de invitar a padres en sitio o de salir.
Llene por favor el cartucho hasta que el borde superior del anillo anaranjado está por debajo de la marca índice que indica 0.1 mililitro.
0
.20
.40
Ensayo Clínico de Inmunización con Vacuna Influenza (INF, gripe)
Hospital Infantil Dr. Robert Reid Cabral

Figure 19. Card for mock 3rd "insurance" injection for group "IM-NS-0.25".


NO administre vacuna a este niño/a.
Aplique una venda aunque no haya inyección.
• Mantenga al niño en la habitación de la inyección por el tiempo que generalmente demora el aplicar una inyección.
• No notifique a los padres o médicos que no se administró una inyección.
• El revelar esta "inyección fingida" puede comprometer el cegamiento del estudio.
• Destruya esta tarjeta antes de invitar a los padres a pasar o a retirarse.
Aplique una venda en el lugar donde generalmente se aplica la inyección: en el muslo izquierdo para niños <12 meses de edad. En el deltoideo izquierdo para niños de 12 meses o mayores.
Ensayo Clínico de Inmunización con Vacuna Influenza (INF, gripe)
Hospital Infantil Dr. Robert Reid Cabral

The outside, independent party who randomly determined the treatment group assignments, as described above, shall insert the cards in accordance with the randomization results into corresponding participant-ID-numbered *allocation group randomization envelopes* (see below). A peel-off "confirmation sticker" with the participant's study ID number and dose (1 = "A", 2 = "B", 3 = "C") will be affixed to the face of each card for attachment to the CRF to verify accuracy in administering the correct type of injection.

10.4.4.4 Allocation Group Randomization Envelopes

The outside, independent party shall prepare four (quadruplicate) sets of *allocation group randomization envelopes.* The envelopes shall be sealed, opaque, and not permit visualization of its contents upon holding up to the light. For each set, consecutive participant ID numbers shall be written, printed, or stickered clearly on the outside of each envelope, e.g., "CDC-ISO-4785 – **001**", "CDC-ISO-4785 – **002**", etc.

Three sets of envelopes, the GREEN, YELLOW and RED sets shall be provided to the Dominican Principal Investigator for use in the study injection room for the FIRST, SECOND, and THIRD study injections, respectively. They shall be marked on their covers "1 - Primera", "2 - Segunda", and "3 - Tercera",

respectively. (Envelopes for the RED set for the "insurance" dose will contain the IM full-dose randomization card shown in **Figure 16** for the two reduced-dose groups IM-NS-0.1 and ID-JI-0.1. RED envelopes for the full-dose group, IM-NS-0.25, having already received the standard 2-dose regimen, will contain a card indicating a mock injection, as shown in **Figure 19**.).

A fourth set of envelopes, the BLUE set, similarly identified on their outside by the participant's study ID number, shall be kept sealed in a secure location (e.g., locked safe) in the custody of the director of the HIRRC. The individual BLUE envelope for a specific participant shall be opened only upon the application of the Dominican Principal or Senior Investigators in the event of a serious adverse reaction for which treatment will depend upon knowledge of the vaccination method (IM-NS-0.25, IM-NS-0.1, or ID-JI-0.1) received by that participant.

The *allocation group randomization envelope* shall be opened by the unblinded injection nurse only when she and the other unblinded study staff (holding assistant) are all in the injection room with the participant of corresponding ID number, and his or her parents have left the room temporarily.

After preparing the proper injection equipment with the proper dose of vaccine, the two staff shall confirm that the delivery device is the same as indicated on the card, its peel-off "confirmation sticker" shall be affixed to the designated box on the corresponding vaccination page in the CRF (parts 7, 11, or 15), and then the card shall be destroyed and disposed in a locked trash receptacle in the same cubicle. After the vaccination, the device used to administer it shall be removed from view and the participant parent invited into the cubicle to sit down and console the child.

10.4.5 Timing of Doses for Each Participant

Upon obtaining informed consent from participants, all participants will receive a dose of influenza vaccine at day 0 (zero) by one of the three dosages and routes of administration (see section 10.1.1). After followup return visits in person to assess adverse reactions scheduled on days 2 and 7, each participant will return for a second dose of vaccine on day 28 (4 weeks), which will be followed again with return visits for adverse reactions on days 30 and 35. A final visit on day 56 (8 weeks) will be scheduled for the third and final blood draw and an "insurance" dose of full-strength vaccine administered by conventional needle-syringe. The days listed above for followup visits and actions have various margins of acceptable deviation, as listed in **Table 6** in section 10.6.1.

10.4.6 Blinding

The study will be observer-blinded. The study investigators, plus the study clinical staff who will assess and record adverse reactions, the laboratory personnel who will process and perform serological assays on the specimens, as well as the participant's parent(s) will all be unaware of the study group into which the participant is assigned. Only the

unblinded *injection nurse* and *holding assistant* will observe the method of injection. None of these unblinded staff will be involved in assessing or recording adverse events in the Case Report or other forms. Parents will be required to remain outside the injection room during the 1 to 2 minutes necessary to carry out the vaccination. The *allocation group randomization cards* (see 10.4.4.3) will be destroyed and discarded at the time when the device is filled for the associated participant. No written record of vaccination dose or route will be maintained in the study clinic.

10.4.6.1 Premature "Breaking" of the Allocation Code

The coding list maintained by the independent outside party and other member(s) of the DSMB (see section 10.4.4) will not be divulged to anyone outside the DSMB, except at the conclusion of the clinical and laboratory aspects of each phase of the study, or on an emergency basis upon request from the U.S. or Dominican Principal Investigators.

In case of emergency requiring immediate knowledge of the method of influenza vaccination for a particular study participant in order to provide appropriate treatment or care, and upon request by the Dominican Principal or Senior Investigator, the blue envelope corresponding to the child's study identification number shall be produced by the hospital director and opened. Such an occurrence shall be reported to the U.S. Principal Investigator.

10.4.6.2 Separation of Parent from Child during Injection

As the careful, credible assessment of adverse events are important for scientific credibility, which determines its usefulness for public health policymakers, it is necessary to ensure the parents are blinded to the treatment group of their child until after the study is completed. Observations and reports by the parents are a significant component of the study. If parents are aware of the vaccination method used, their reports my be subconsciously biased, and they may inadvertently unblind the investigators through a casual comment or question, further undermining objectivity of the study findings.

Unlike other vaccine trials where the investigational product and the control product or placebo are both administered in a syringe, in this study the method of administration itself is under investigation and not easily concealed. The psychological discomfort of both child and parent that may be caused by the brief 1-to-2 minute separation are deemed an unfortunate but necessary requirement for the study, for which the parents will be thoroughly informed in advance and their voluntary consent required to participate. Alternative blinding methods that might have permitted the parent to remain in the injection room with the child were considered, but deemed unsatisfactory:

• **Multiple injections on each visit.** This method has been used in prior studies by others, in which every patient gets injected on every visit with both methods of

injection, only one of which contains the drug of interest, and the other saline placebo. In our study, this would require administration of a needle-syringe IM (intramuscular) injection and a jet injector ID injection for every injection visit. An unblinded pharmacist or other health worker outside the parent's and investigator's view would fill only one of these with the influenza vaccine, according to the randomization group, and the other device with a placebo of similar appearance.

Although perfectly controlled and thus valid from a scientific perspective, this method of blinding was rejected as causing twice as much psychological and physical discomfort than otherwise needed to satisfactorily answer the research questions.

• **Blindfolding and earmuffing the parent in the injection room.** Blindfolding the parent, who could be visible to the child in the room, would not be sufficient as a blinding method, as the jet injector makes a "poof" of noise. Masking that sound with electrically-powered, noise-cancelling headphones playing masking music into the parent's ears was considered. Alternatively, a simultaneous jet injector could be fired into the air by a third unblinded health worker, so that all parents heard the noise, regardless of study group allocation.

Blindfolding (with or without earmuffing) was rejected as demeaning to the parent and potentially frightening to the child. Simultaneous firing of a jet injector for those receiving needle injections was rejected as (1) labor-intensive, (2) potentially ineffective for blinding if needle injection (and patient recoil) and jet firing are not exactly simultaneous, (3) time-consuming, and (4) prone to medical error, such as inadvertent confusion of a jet cartridge filled with tap water for a mock injection into the air with a cartridge to be used to administer actual vaccine to a child in the ID-JI-0.1 group.

Standard practice in the Dominican Republic is for parents to be absent during certain pediatric procedures, particularly delicate or painful ones, such as phlebotomy and tympanocentesis, for which informed-consent otitis media studies are now being performed with parents absent from the treatment room.

Beyond the need for blinding, other reasons to avoid the parent holding the child during the injection have been provided by the U.S. Naval Medical Research Center (NMRC) pediatrician, IRB member, and principal investigator of a prior study of the Biojector with investigational ID spacer, Dr. Judith Epstein.[Epstein2002] Since the sound of the injector might cause the parent to release firm hold on the child, it was suggested that the child should be restrained instead by a trained nurse aware of the manufacturer's instructions that the limb and injection not move in relation to each other during the 1/3 second that the injection requires, to avoid any risk of laceration.

Other informed-consent trials with parents excluded from the vaccination room have been performed when the investigational vaccine could not otherwise be

blinded. These included a recent trial of the intranasal FluMist® influenza vaccine squirted into the nose, compared to an injected vaccine.[King2006] Other influenza vaccine studies have been done in children without parents present for the vaccination, and with such absence duly consented in advance.[Khan1996, Rudenko1993, Tanaka1993]

The consent form advises the parents of the need to be separated from the child, just outside the door of the injection room, for the estimated 1 to 2 minutes to prepare and administer each of the first three study injections. The consent form permits them the opportunity freely to withdraw consent in advance of the study (and thus not to enroll), or to withdraw at any time during the study.

Phlebotomy. The presence or absence of the parents during the required three blood collections in this study would not affect blinding of the study. In keeping with common pediatric practice in the Dominican Republic and in most U.S. medical facilities, the parents will be requested, for their own peace of mind, to remain outside the room where phlebotomy and similar uncomfortable or frightening diagnostic (e.g., lumbar puncture) or therapeutic (e.g., tympanocentesis, surgery) treatments are performed. However, in this study, if the parents request to be present during phlebotomy, they will be permitted to do so, and this option is mentioned in the consent form.

10.4.6.3 Caching of Immediate Injection Site Observations by Nurses

To prevent unblinding of the physician investigators to the method of injection used, the immediate reactions observed by the unblinded injection nurses at the injection site (e.g., drop or flow of blood or wetness) for investigational doses 1 (Part 8, questions 1 to 8) and 2 (Part 11, questions 1 to 8) will be noted on separate colored pages (green 12enf and yellow 16enf, respectively) of the Case Report Form. For reasons of convenience, onto these removable colored pages will be moved other data items also completed by the nurses for dose 1 on page 11 (Part 7, questions 7 to 11) and for dose 2 on page 15 (Part 11, questions 7 to 11). For the injection, the nurses will separate and complete these pages and place them in a closed file in the injection room. After the investigational period of observation after dose 2, and after the physician investigator has entered all related assessments into the C.R.F. for a particular participant, these colored pages will be retrieved for insertion back into the rest of the C.R.F. and copied for forwarding to the CDC investigators for data entry and analysis.

10.4.7 Prior and Concomitant Therapy - Other Vaccinations

No other routine or investigational vaccines will be administered within 28 days before or after either of the study's investigational **INF** doses 1 or 2. As per the study exclusion criteria (see paragraph i. in section 10.3.2), otherwise eligible participants will not be recruited if they have received in the prior 28 days any routinely recommended vaccinations of the current Dominican Republic public immunization policy, or are due

to receive them in the following 56 days. The recommended Dominican immunization schedule indicates vaccines at 6 months (**POL_{OPV}**, **DTP_w-HBV-HIB**), 12 months (**MMR**), and 18 months (**POL_{OPV}**, **DTP_w-HBV-HIB**) of age.[SESPAS]

This restriction thus excludes infants "behind schedule" and needing to receive on the day of first interview protection against tuberculosis, polio, diphtheria, tetanus, pertussis, hepatitis B, *Haemophilus influenzae* type b, measles, mumps, or rubella. It also creates three gaps in the intended ≥ 6 -to- <24 months age range of recruited participants for this study. The first gap would be ages <7 months (unless the participant received his or her third **POL_{OPV}** and **DTP_w-HBV-HIB** doses at ≤5 months); the second gap from >10 -to- <13 months; and the third gap from >16 -to- <19 months.

If, in the opinion of the Dominican investigators, a child may benefit by immediately receiving another vaccine during the 28-day-period after either of the study's influenza vaccine doses (e.g., because of exposure to meningitis or another vaccine-preventable disease), such other vaccines should be administered by the appropriate authority and duly noted on the Case Report Form, part 20.

10.4.8 Treatment Compliance and Participant Incentives

Adherence of study participants to the vaccination and visit schedule will be supervised by the study nurses and senior clinical staff. A total of 8 visits are planned, on days 0, 2, 7, 28 (4 weeks), 30, 35, 56 (8 weeks), and 8 months (see **Table 5** in section 10.6.1 for details).

Parents will be encouraged to return with their infant, diary form, and related items for scheduled clinic visits through the provision of modest incentives, which may include:

- round trip taxi fares from and to home
- voucher for lunch or snack in the hospital snack bar
- folder and pencils for storing and marking take-home materials (diary form, measuring tape and template, adhesive thermometers)

For those children who do not return to the clinic as scheduled, study health workers will collect the information by telephone, reschedule the visit, and/or make home visits on the days following the missed scheduled visit.

10.5 Efficacy and Safety Variables

10.5.1 Efficacy

10.5.1.1 Immunogenicity

In this trial, the surrogate for measuring actual field efficacy (protection from actual disease through natural exposure to wild virus) will be the immune responses generally considered predictive of such protection.

10.5.1.2 Primary Efficacy Variable

Seroconversion (SC) is the primary efficacy variable. It is defined as the development of an inverse hemagglutination inhibition (HI) titer of ≥40 on *followup* serum collected one month after the second vaccine dose among initially-seronegative participants (undetectable inverse titer of <8 on *baseline* day 0); OR, for participants with a detectable *baseline* titer, the development of a *followup* HI titer which rises ≥4-fold. (Usually analyzed as a proportion among participants.)

10.5.1.3 Secondary Efficacy Variables

Secondary efficacy variables are:

Seroprotection (SP), defined as the development of an inverse HI titer of ≥40 on *followup* serum collected one month after the second vaccine dose, regardless of evidence of immunity at *baseline*. (Usually analyzed as a proportion among participants.)

Mean geometric increase, defined as the ratio of the geometric mean titers (GMT) of all participants on *followup* HI assay divided by the GMT of HI assay on the same participants at *baseline* (excludes participants with baseline titers lost to followup). (For example, a mean baseline inverse GMT of 18 and followup of 81 would have a mean geometric increase of 4.5.)

Dose 1 seroconversion, defined according to the seroconversion criteria above one month after only the first dose of influenza vaccine, before receiving dose 2.

Dose 1 seroprotection, defined according to the seroprotection criteria above one month after only the first dose of influenza vaccine, before receiving dose 2.

Dose 1 mean geometric increase, defined according to the mean geometric increase calculation above after only the first dose of influenza vaccine, before receiving dose 2.

10.5.1.4 Subsequent Specimen Storage and Laboratory Assay(s)

Upon separate written signature in the informed consent form, leftover serum not needed for study assays may be retained in indefinite long-term storage. Such consent may be provided or declined without affecting consent for participation in the study. In accordance with the informed consent form, and at the discretion of the sponsor and upon identification of required resources and interest, any such serum remaining from the study after completion of the testing described above may be tested by other laboratories by additional assays for purposes of comparison and correlation with the results obtained in the laboratory testing described herein.

10.5.2 Safety

Participants will be observed and followed prospectively after both dose 1 and dose 2 of the investigational INF vaccinations for the occurrence, frequency, and severity of immediate and delayed local and systemic reactions and other adverse events.

10.5.2.1 Local and Systemic Reactions

Reactogenicity will be assessed from clinical staff and parental observation, physical examination, and measurement of immediate and delayed local reactions at the sites of injection (i.e., drop or flow of blood, tenderness, erythema, swelling, hematoma, induration, warmth, nodule, crying upon movement of limb, limb swelling, limb circumference at midpoint) and of immediate and delayed systemic reactions (i.e., anaphylaxis, fever, sleepiness, irritability, unusual or inconsolable crying, vomiting, diarrhea, convulsions, change in eating habits,), among non-prompted other adverse events to be solicited.

Temperature. Axillary temperatures will be measured and recorded by nursing staff and parents from reusable, disposable, 2-day-wearable adhesive thermometers (TraxIt® McKenzie2003, MedicalIndicators2004 http://www.medicalindicators.com/html/traxit.html) at clinic visits and at home. The TraxIt® will be applied by the nurse in one axilla on day 0, and replaced by the parent with a new one in the opposite axilla on days 2, 4, and 6. Parents will be trained upon recruitment in daily reading of the thermometer from days 0 through 7, and to record the temperatures, as well as any use of analgesic/antipyretic medication and other items prompted on the diary card. An additional TraxIt™ will be provided to the parents for use to record temperatures, as needed for suspected fever or illness which may occur on days 9 through 27 after each investigational vaccination.

Local Reaction Size Measurements. Parents will be trained upon recruitment in (1) using supplied plastic template cards to measure the largest diameters of any erythema and induration for the vaccine injection site in the left thigh (age <12 months) or left deltoid (age ≥12 months), (2) using supplied [infant head] circumference tapes to measure the circumference of the thigh or upper arm at its

midpoint, and (3) filling out diary cards to record these and other prompted and unprompted local and systemic reactions. During clinic visit interviews and telephone followup or home visits, as needed, nurses will assist parents in completing the diary cards.

Photography of Injection Site. On days 2 and 7 after vaccination, the injection site will be photographed in color with a digital camera. The field of view will include a scale to assess reaction sizes, and a label with the participant's identification number, initials, and the current date. In case of unexpected or unusual local reaction during the 30-60 minute period after vaccination, such as frank bleeding or laceration, the injection nurses will notify the physician investigator, who will photograph it (after first initiating medical treatment, if necessary, and subsequently entering details in the adverse events section of the Case Report Form). Any visible local reactions persisting on day 28 after vaccination will be photographed, as well. In addition, upon recruitment and signed consent, a digital photo will be taken of the mother and participant for pasting into the Case Report Form to assist in preventing entries into the wrong form. For privacy protection, this photo will be removed before the CRF is copied for archival purposes at the study site and then mailed to the sponsor.

10.5.2.2 Safety Variables and Monitoring

10.5.2.2.1 Definitions

Definitions and classifications for specific adverse events to be reported from this study will conform, as much as practicable, with those in draft development or finalized by the Brighton Collaboration (http://brightoncollaboration.org).

- An ***adverse event*** (AE) is defined as any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, at any dose, or placebo. It does not necessarily require a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. This definition includes intercurrent illnesses or injuries, and exacerbation of pre-existing conditions.

- An ***expected* AE** is one previously known or anticipated as a result of (a) the interventions and interactions used in the research, (b) the collection of identifiable private information for the research; (c) an underlying disease, disorder, or condition of the human subject; and/or (d) other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject.

- An ***unanticipated* AE** is an unexpected occurrence or frequency of a type not previously known or commonly identified to result from (a) the

interventions and interactions used in the research; (b) the collection of identifiable private information under the research; (c) an underlying disease, disorder, or condition of the human subject; and/or (d) other circumstances unrelated to the research or to any underlying disease, disorder, or condition of the subject.

- An ***unanticipated problem that does not result in an AE*** is an occurrence that otherwise may have increased the risk of physical, emotional, social, or legal harm to the participant, when no actual harm was detected. Examples include (1) breaches in confidentiality if participant identifying information is improperly disseminated, lost, or stolen; (2) administration of an expired, recalled, or improper dose (too little, too much) of either investigational or routine medication; (3) exposure of the participant or family member to an active tuberculosis patient while in the study waiting room.

10.5.2.2.2 Severity

The severity of events reported on the AE Case Report Form (CRF) will be classified by the investigators as follows:

- **Mild**: An adverse experience that the subject tolerates easily, causing a minimum of uncomfortability that does not interfere with daily activities.
- **Moderate**: An adverse experience of sufficient uncomfortability to interfere with normal daily activities.
- **Severe**: An adverse experience that prevents normal daily activities. In a young child, such adverse effect might, for example, impede attendance at school/pre-school/day care, and/or cause parents/teachers to seek medical attention.
- **Life-threatening**: An adverse experience with immediate risk of death.

10.5.2.2.3 Seriousness

A ***Serious Adverse Event*** is defined as any untoward medical occurrence which… :

- Results in death.
- Is life threatening (i.e., the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred).
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity, (i.e., the event causes a substantial disruption of a person's ability to conduct normal life functions).
- Is a congenital anomaly or birth defect.
- Is an important and significant medical event that, based upon appropriate medical judgment, may jeopardize the subject and may require

medical or surgical intervention to prevent one of the other outcomes defining serious. [1]

1. Medical and scientific judgment should be exercised in deciding whether an adverse event not satisfying any of the first five bullets above is "important and significant" and thus should be classified as a Serious Adverse Event (SAE) and reported by expedited method. Examples of such events that would usually be considered important and significant include (a) allergic bronchospasm requiring intensive treatment in an emergency room or at home, (b) bleeding disorders or (c) convulsions that may not result in inpatient hospitalization, (d) the development of drug dependency or drug abuse, or (e) a diagnosis of cancer. Examples of medical events that would usually NOT be considered SAEs, if deemed unrelated to the vaccine, which resolve upon standard treatment with drugs or other therapies, and which do not result in any of the outcomes described in the first five bullets of the SAE definition above, would usually include common acute childhood conditions such as (f) otitis media, (g) upper respiratory infection, (h) influenza and similar illness, (i) mild reactive airway disease or asthma, (j) diarrhea, (k) impetigo, and (l) intestinal parasitosis. All such events not considered SAEs would still be reported routinely as adverse events (AEs) on the Case Report Form.

It should be noted that a *severe* Adverse Event need not be *serious* in nature and that a *serious* Adverse Event need not, by definition, be *severe*.

10.5.2.2.4 Relationship to Vaccine

The relationship of the study treatment to an AE will be determined by the investigator based on the following definitions:

- ***Not related*** - The AE was definitely not caused by administration of the study vaccine(s).

- ***Possibly Related*** - The administration of the study vaccine(s) and the adverse experience are reasonably related in time AND the adverse experience could equally be explained by causes other than exposure to the vaccine(s).

- ***Probably Related*** - The study treatment and the AE occurrence were reasonably related in time, and the AE was more likely explained by exposure to the study product than by other causes.

10.5.2.2.5 Reporting

Any serious AE, whether or not considered study related, will be reported immediately (within 24 hours) to the U.S. PI. The individuals to be contacted at CDC for notification of a serious AE are the U.S. PI and co-investigator identified in sections 8.1.1 and 8.1.2 above, who will in turn notify the U.S. IRB and provide courtesy notification to the vaccine manufacturer.

Any serious or unanticipated AE must also be reported to the Dominican IRB in a timely manner. Adequate documentation must be provided to CDC showing that the IRB has been properly notified. Any medication or other therapeutic measures used to treat the event will be recorded on the appropriate case report form (CRF) page(s) in addition to the outcome of the AE.

10.5.2.2.6 Premature Discontinuation of Participants; Followup

A genuine effort must be made to determine the reason(s) why a subject fails to return for the scheduled visits. If the subject's parent/guardian is unreachable by telephone, a study staff member should be sent to find him or her and encourage contact with the clinic. This information should be recorded in the appropriate section(s) of the Case Report Form, including Part 20 – Comments.

AEs will be monitored until resolution or, if the AE is determined to be chronic, a cause is identified. If an AE remains unresolved at the conclusion of the study, a clinical assessment will be made by the investigator whether continued follow-up of the AE is warranted.

10.5.2.3 Determination of Followup Period for Adverse Events

For influenza vaccination, 28 days of followup is relatively conservative and would catch all but the rarest of complications. The common local and system reactions following influenza vaccination occur within the first two days.[CDC2005] The day 2 visit was included particularly to permit investigator observation of such reactions. Nearly all would occur by the day 7 visit, and certainly by the day 28 visit.

As noted in the informed consent form, a very, very rare complication of influenza vaccination is the nervous system Guillain-Barré syndrome. Section 10.4.2.7 discusses the risk of GBS in more detail and its usual window of onset for it after influenza vaccination.

The 28 days of followup after the each investigational doses 1 and 2 would pick up the great majority of this extremely rare condition.[Schonberger1979, Haber2004, Lasky1998] Moreover, with the "bonus" 4th influenza vaccination 6 months after the "insurance" dose 3, virtually all GBS which might rarely occur as a result of the first 2 or 3 doses of the study would come to the investigator's attention.

The Completion Certificate and Bonus Vaccination Reminder for the parents upon their child's graduation from the study (on day 56 at the time of the 3rd "insurance" dose) reminds them to bring the child back 6 months later for the "bonus" dose for next influenza season, as well as to contact the doctors for any unusual illness or condition which may occur in the child before that revisit.

10.5.3 Appropriateness of measurements

The measures of efficacy to be used in this study are standard, i.e., widely used and generally recognized as reliable, accurate, and relevant (able to discriminate between effective and ineffective agents). The measures of safety used in this study are routine clinical and laboratory procedures

10.5.4 Pharmacokinetics

No pharmacokinetic studies are intended in this project, as they are not relevant to most vaccine research.

10.6 Study Procedures

10.6.1 Time and Events Table

The sequence and chronology for conducting clinical and diagnostic activities is summarized in **Table 5**. Allowable variations from the planned schedule of followup visits are detailed in **Table 6**.

Table 5. Time and events

Procedure / Test	days* 0	2	7	28	30	35	56	56d + 6 months
SCREENING – recruitment, informed consent, medical history	✓							
EXAM – weight, height, limb circumference, temperature, local/systemic reactions	✓	✓	✓	✓	✓	✓	✓	
BLOOD SAMPLE for hemagglutination inhibition serologies	✓			✓			✓	
VACCINATION with **INF** vaccine by blinded dose and route	✓			✓				
PHOTOGRAPH of **INF** injection site, closeup with scale	‡	✓	✓	‡	✓	✓		
REVIEW and discuss parent diaries; collect completed ones		✓	✓	✓	✓	✓	✓	
"Insurance" VACCINATION 0.25 mL **INF** IM by N-S†							✓	
"Bonus" booster VACCINATION 0.25 mL **INF** IM by N-S; inquiry on intervening adverse events								✓

* See next table for acceptable windows for scheduled visit and followup dates.
† Groups **IM-NS-0.1** and **ID-JI-0.1** receive full dose. Group **IM-NS-0.25** receives mock injection.
‡ Photography only if any injury or persistent local reaction is visible to be photographed.

Table 6. Acceptable windows for followup visits

	Intended day since 1st	Day to be scheduled	Permitted variation	Earliest permitted	Latest permitted day	Latest weeks

Visit no.	vaccination	since last vaccination	from scheduled day	day since 1st vacc.	since 1st vacc.	since 2nd vacc.	since 1st vacc.
1	"Day 0"	0					
2	"Day 2"	+2	+/- 1 day	1	3		0.4
3	"Day 7"	+7	+/- 1 day	6	8		1.1
4	"Day 28"	+28	+ 14 days	28	42		6.0
5	"Day 30"	real 2nd-dose day +2	+/- 1 day	29	45 *	3	6.4 *
6	"Day 35"	real 2nd-dose day +7	+/- 1 day	34	50 *	8	7.1 *
7	"Day 56"	real 2nd-dose day +28	+ 28 days	56	98 *	56	14 *
8	8 months	+6 months	+3 months	8 months	98 + 9 months	56 + 9 months	53 *

* Assumes 2nd vaccination at latest permissible day (42) since 1st vaccination.

10.6.2 Training of Study Personnel

Training for Dominican study personnel will be conducted in two stages lasting one week each. In addition, U.S. investigators and trainers from the jet injector manufacturer will be present during relevant periods of this training. Dominican study staff include 5 registered nurses, 3 auxiliary nurses, 3 physicians, one resident in pediatrics, one data/files manager, one laboratory professional, one laboratory technician, and 3 auxiliary nurses.

10.6.2.1 Stage 1: Logistics and Practice

Monday in the first training week will be devoted to study logistics, making sure that all the necessary equipment and materials are in place and functioning, and telecommunications are working. Principal and senior investigators and co-investigators will inspect each physical location to verify all study materials and equipment all are in place, each is functional, and ready to start the study (See **Table 7**).

Table 7. Required Study Supplies and Equipment

Study Center Office	1. Lockable entry door with "Vaccination Study Center" identification sign viewable from hallway
	2. Sufficient electrical outlets to supply below equipment
	3. Lockable Filing cabinet
	4. 4 chairs
	5. 1 desk
	6. Direct line and/or PBX extension telephone

	7. Laptop computer, including cables, charger
	8. Printer/Scanner/Copier/Fax
	9. Color photo printer, and cables
	10. Digital camera, including spare memory chip, batteries, charger, cables
	11. Registration card photo cutter and laminator and supplies
	12. Regular waste disposal container
	13. Individual participant files by ID number for storing relevant active and completed forms (i.e., green, yellow, and red randomization envelopes with attached blood specimen peeloff numbering stickers, signed Consent, 0-7 and 7-28 Diaries doses 1 and 2, CRF, Participant Identification, Serious Adverse Events, Data Clarification)
	14. Daily appointment book and/or wallboard for logging and tracking followup appointments
	15. Study files: Spanish Protocol, English Protocol
	16. Study files: Study Staff Responsibilities, Signature Registry and Log
	17. Study files: Current and completed Enrollment Log/Participant Inscription sheets
	18. Supply of blank Serious Adverse Events forms
	19. Supply of blank Appreciation/Bonus Dose Reminder sheets
	20. Supply of blank Participant Registration cards
	21. Supply of blank Appointment Reminder slips
	22. Blank copier paper
	23. Blank fax transmission cover sheets
	24. Supply of TraxIt skin-applique thermometers
	25. Regular thermometers
	26. General office supplies, including pens, pencils, paper clips, rubber bands, blank CD-ROMs
Waiting Area	27. At least 10 chairs available
Study Center Injection Room	28. Emergency resuscitation equipment, including epinephrine and 1cc syringes, ventilatory bag&mask of appropriate pediatric sizes for manual respiration
	29. Insulated cold box with wet ice containing day's supply of opened and spare vaccine vials, and virus specimen collection kits (return contents to Microbiology refrigerator at end of day)
	30. Writing table
	31. Exam table
	32. 2 stools or chairs

	33. Restraint board
	34. Regular waste disposal container
	35. Medical waste disposal container
	36. Sharps waste disposal box
	37. Examination gloves, various sizes
	38. Alcohol, alcohol wipes
	39. Gauze
	40. skin adhesive tape
	41. Latex-free bandages (e.g., Band-Aids®) for covering injection site
	42. Butterfly needles, various gauges
	43. Blood collection tubes, various sizes, red top for serum
	44. Waterproof marker pens for specimen vessels
	45. Sterile Alaris SmartSite® vial adaptors multi-use access ports for vaccine vials
	46. Syringes, 3-5 mL
	47. Syringes, 10 mL
	48. Syringes, 1 mL, tuberculin/vaccination type
	49. Biojector devices and spare CO2 cartridges, instructions
	50. Sterile Biojector jet injector cartridges
	51. Identification stickers for injection site photography
	52. Shredder for destroying randomization cards
	53. Virus collection specimen peel-off numbering stickers
Clinical Investigations Consultant's Office	54. Scale
	55. Height/length measuring board
	56. Furniture
	57. Week's supply of blank Consent - Additional Information handouts
	58. Week's supply of blank Informed Consent Forms
	59. Week's supply of blank Participant Identification Forms
	60. Week's supply of blank Enrollment Log / Participant Inscription sheets
	61. Week's supply of blank Case Report Forms
	62. Week's supply of blank Parent Diary Forms Days 0-7
	63. Week's supply of blank Parent Diary Forms Days 7-28
	64. Week's supply of blank Data Clarification Forms
	65. Week's supply of blank Serious Adverse Event Forms
	66. Week's supply of adhesive thermometers
	67. Week's supply of circumference tapes
	68. Supply of skin-measuring ruler circle templates
	69. Supply of TraxIt skin-applique thermometers
	70. Regular thermometers
	71. Identification stickers for injection site photography

	72. Week's supply of parent take-home notebook/satchel
Hospital Director Office	73. Sealed blue envelopes for emergency opening opening containing study group allocations for each participant ID number.
Microbiology Laboratory	74. Freezer at -70° C for serum and virus specimen storage – protected by emergency backup generator
	75. Refrigerator at 2-8° C for storage of vaccine (and unused virus collection kits, and influenza rapid assay test kits, if needed) – protected by emergency backup generator
	76. 15 kW backup generator for above items with automatic switching and start control equipment.
	77. Sufficient fuel in backup generator tank for 72 hours operation
	78. Centrifuge for serum
	79. Study vaccine supply in above refrigerator
	80. Study virus collection kits supply in above refrigerator (if indicated)
	81. Study rapid influenza test kits supply in above refrigerator, if indicated
	82. Designated space in -70° C freezer for serum cryovials and virus collection specimens
	83. Pipettors and pipette tips
	84. Cryovial tubes and holding racks
	85. Storage/freezing boxes for cryovial tubes
	86. Cold packs for shipments
	87. Miscellaneous laboratory supplies and equipment
Study Storage Room	88. Study supplies in reserve and excess which do not fit into above rooms
	89. Cold shipping containers for overseas transport of specimens
Dept. of Infectious Disease / Fundación Dominicana de Infectología office	90. FDI IRB files and documents
	91. Study administrative files, contracts, etc.

Stage 1 Monday will also be devoted to general orientation of the physician investigators and study nurses for the study and its timelines, processes, and forms, in lecture style with handouts, questions and answers, and discussion. Repeat morning and afternoon sessions may be used for staff on shift duty during the other session.

Stage 1 Tuesday and **Wednesday** will be devoted to training the physician investigators who will administer the informed consent, do clinical assessments, and complete the CRF and other data forms. It will also include the training of the nurses who will administer the blinded injections and manage the flow of participants, paper, and specimens.

With social worker observation, physicians will explain the informed consent to one another in pairs, with one serving as mock parent. Upon signatures, the mock parent will later complete each of the 0-7 and 7-28 day diary forms. Later, the pair will reunite to continue mock revisits on day 2, day 7, and day 28, The completed mock Diary forms will be used for physician-"parent" interview and data entry into mock CRFs. Afterwards, all investigators and mock parents will review the process in an interactive session seeking discussion, questions, answers, and suggestions.

The study nurses will undergo training by principal/senior investigators in the blinding process and related security issues. This will include the process of opening the random allocation cards, preparing the injection equipment, shredding the card, and administering the injections.

Representatives of the Biojector manufacturer will demonstrate proper jet injector technique, which will be practiced by study nurses. Proper needle-injection technique for children above and below 12 months will also be taught.

On **Phase 1 Wednesday**, laboratory personnel will conduct a session for physicians and nurses regarding laboratory procedures for the collection, labeling, processing and handling of blood specimens from all participants, and nasopharyngeal swabs from those with influenza-like illness. Demonstrations and training will occur in collecting nasopharyngeal swabs, placing them into swab containers and virus collection tubes, labeling with mock sticker, and transporting to the laboratory for processing and placement into the freezer.

Phase 1 Thursday and **Friday** will be devoted to ensuring no gaps in the flow of parents, patients, forms, supplies, specimens, and data. The sponsor representatives, physician investigators, and study nurses will "walk-through" the entire physical and administrative process with mock parents using participant number "000" to work out the patient flow and paperwork entries from ground floor immunization clinic to 2nd floor study center rooms.

The first walk-through will be for a mock day 0 recruitment from the immunization clinic for consent and vaccination with dose 1. Walk throughs will be repeated for a mock day 2 visit, then a day 7 visit, then a day 28 visit, then a day 56 visit, then a day 56 +6 months visit. Investigators will observe the process. Unofficial samples of all study forms will be completely filled out as if real, including opening of mock random allocation envelopes. Mock injections will be made into the air,

mock blood tubes labeled with mock stickers will be brought to lab, etc. Real photographs and printed out of mock Parent/Child for registration card and CRF, and of injection site reactions.

Volunteers for the day 0 walk-through will be sought from among parents waiting in the immunization clinic (or among other hospital staff or volunteers, if engaging parents are not possible). Volunteer parents will be compensated for their time. The vaccination or procedures for which they came to the clinic will be completed first.

Volunteer parents and children for the mock walk-through will be directed to the study clinic where the the study will be explained and they will read and consider the informed consent and additional information handouts, as if in a real situation. After a mock signing, all other relevant study forms will be filled out (e.g., CRF, Diary dates, etc.). Parents will be trained in taking measurements and filling out the diary forms. That will be the extent of participation by these parent volunteers in the study walk-through. Unless obviously ineligible, they should be invited to return in the future for actual recruitment into the study.

The rest of the simulation exercise will use three hospital staff, who will be given baby dolls to represent study participants. They will "arrive" at the study center and be directed to wait outside, as would actual participants. They will be invited to enter for processing, photographs, and issuance of mock registration cards and next appointment reminders. They will wait outside until called in for blood collection and vaccination.

The study nurses will take custody of the dolls for mock venipuncture and mock "vaccination" according to mock randomization envelopes. One of the dolls will be presumed to have influenza-like illness and undergo mock nasopharyngeal swabbing and specimen collection, as well. Specimens will be labeled with mock stickers and brought to the laboratory for mock processing. Laboratory personnel will simulate processing them (labeling, separation, aliquoting) and storing in the -70° C freezer. The steps in the simulation will be timed.

Week 1 Friday afternoon will feature a debriefing session by sponsors and investigators to assess readiness and preparedness. If positive, training phase 2 will begin the following Monday, with or without using the intervening **Saturday** and/or **Sunday** to resolve any weak points. If readiness and preparedness are not sufficient, training phase 1 will be continued into the next week.

10.6.2.2 Phase 2: Pilot Actual Recruitment and Study Initiation

On training phase 2, on a **Monday** and **Tuesday**, the actual study will begin in a slow-motion pilot with recruitment at a reduced rate. Only one volunteer parent/participant will be recruited into the study each morning and each afternoon of **Monday** and **Tuesday** only, with actual blood collection, blinded vaccinations,

laboratory processing and all other study procedures. At any time when conditions dictate, the investigators may suspend further recruitment until readiness resumes. These four pilot phase 2 participants will return for their 2 day visits on **Wednesday** and **Thursday**, respectively, and for their remaining visits in future weeks, as per protocol.

Extra time will be spent assessing the parent diaries for correctness of completion and revising the parent-training process to overcome any problems detected. Each afternoon of the training phase 2 week, the investigators and other study personnel will meet to discuss problems and develop solutions. On **Friday**, a decision will be made whether and how much to increase the recruitment rate for the following week, and to add the remaining vaccination day of Wednesdays.

10.6.3 Screening and Recruitment

Recruitment and screening of participants will occur in the following steps. There will be no posters or public announcements.

A. The nurses who staff the Healthy Children and Vaccination Service [immunization clinic] of the hospital will identify eligible children among those in their waiting room by examining their visitor log and questioning parents. Eligibles will be patients or their accompanying siblings who are age 6 to 24 months and up-to-date with the national immunization schedule. (These nurses, in alternating morning and afternoon shifts, will serve as the study nurses who will handle logistics in the study center and will be trained to administer unblinded the study vaccinations.)

B. Parent(s) or guardian(s) (hereafter referred to by the singular "parent" but including the plural) of potential eligibles will be invited to consider participation of their child in an influenza vaccination study. The parent will be informed that non-interest or non-participation will not in any way affect the services they came to receive in the clinic.

C. If the parent expresses interest in the study, or asks technical questions that the nurses cannot easily answer, the parent and child will be escorted or directed to the Clinical Investigations Consultant's office, room 33, second floor.

D. There, the investigator physician will fully explain the study verbally, as detailed in the study information document, which the parent will be requested to read, as well.

E. If participation is declined, the parent will be thanked for their time and interest and escorted or directed with the child back to the ground floor Healthy Children and Vaccination Service [immunization clinic], where they will be attended to, if needed, or resume their original position in the queue.

F. If participation is desired, the investigator will interview the parent to determine that the child fulfills all the inclusion criteria and none of the exclusion criteria for participation. If not currently eligible due to age or recent vaccination, a return date

will be selected for their return, and the parent and child escorted or directed back to the immunization clinic, where they will resume, if needed, their original position in the queue. If permanently ineligible, this will be explained to the parent, who will be thanked for her time and interest and escorted or directed back to the immunization clinic, where they will resume, if needed, their original position in the queue.

G. To ensure compliance with the inclusion and exclusion criteria of the protocol and Part 4 of the Case Report Form (CRF), duplicate verifications will be required. First, the physician investigator who originally collects the demographic and clinical history in CRF Parts 2 and 3 will certify eligibility in question 30 of the CRF Part 4. Second, a different physician investigator will review the data to independently verify and certify eligibility of the child for the study in question 31, and duly sign in field 32.

10.6.4 Informed Consent Process

The informed consent process will occur in the following steps:

A. The investigator will inform parents that participation requires their written consent and the content of the consent form will be explained verbally. The parents will be requested also to read the consent form themselves, as well. If they agree to participate, they are requested to sign and date it.

B. Parents will be given the opportunity separately to consent or decline long-term storage of leftover, unused specimens for future testing (sections 7.3 and 10.5.1.4). Declining long-term storage and future testing shall not bar participation in the study if otherwise consented.

C. Parents who do not wish to participate in the study or to sign its consent form will be thanked for their time and interest and escorted or directed back to the Healthy Children and Vaccination Service [immunization clinic], where they will be attended to, if needed, or resume their original position in the queue.

10.6.5 Monitoring of Informed Consent Process

A social worker from the office of social work at the HIRRC will silently observe the first two weeks (or longer, as needed) of the informed consent process, in order to identify any difficulties or misunderstandings. He or she will request voluntary informal interviews with a convenience sample of parents upon their completion of day 0 activities, or upon their return on days 2 or 7, to explore the parents's comprehension of the informed consent, and any lingering questions. The social worker will meet three times weekly during this period, or more frequently as needed, with the Dominican Investigators to provide feedback on any gaps or weaknesses perceived in the informed consent process, so that its verbal explanations and other processes may promptly be revised or improved, if necessary.

10.6.6 Parent Training Procedure

Both in the Dominican Republic and the U.S., the filling out of diaries by parents to record vaccination-related observations at home are a common and accepted procedure. In the years 2002 through 2006 alone, the HIRRC itself collaborated in three multi-center studies with international pharmaceutical manufacturers on studies meeting current good clinical practice (cGCP). These required Dominican parents to record information daily at home about their child's illness following treatment for recent onset of middle ear infection (acute otitis media). In the United States, controlled, clinical trials of influenza vaccine have used parental diary cards to record information at home.[Edwards1994, Belshe1998, Belshe2000]

Parent training will occur, as follows:
A. While the physician investigator is conducting the physical examinations required in the CRF, he or she will explain in the parents presence how to perform those methods which they will repeat at home and record in the Diary form.

B. Before handing the Diary form to the parent, the investigator (or study nurses earlier) will prefill fields in the diary form for participant initials and ID number, study center telephone numbers, and the dates and days of the week at the top of Diary columns for recording reactions.

C. Using sample forms, the investigator will show and practice with the parents how to complete the Diary form. On the Day 7 visit, similar filling out and training will occur for the 7-28 day Diary form.

D. Specific skills the investigators and/or nurses will teach the parents include correct application, reading, and removal of the skin appliqué thermometer, use of the tape measure for limb circumference, and reading local reaction sizes using the plastic ruler/template.

E. Parents will be required to demonstrate actual use of these devices, and proper recording of the results in the form, in order to verify their understanding. Mistakes will require additional explanation until the parents can complete the form correctly, or the investigator determines that the parents are unable to do so and are excused from the study.

F. Parents will be provided with a study registration card with a photograph of the parent and child (to reduce the chance of mistaken identity on later visits). They will also receive an appointment card on which the days and hours of their next appointment will be written. Both cards will contain the telephone numbers to be contacted at any hour if any questions or concerns arise.

G. Parents will be provided with a take-home, closable notebook and/or satchel to carry their registration and appointment cards, the ruler/template and circumference tape, additional skin thermometers for replacing every two days, a signed copy of the informed consent and its accompanying additional information handout, plus the

parental Diary form to be used until day 7 (on that day, it will be replaced with another for the period from day 7 to 28).

H. The importance will be explained to the parents for their correctly completing the Diary form each day, and bringing the child back on days 2 and 7 and 28 after vaccination to be evaluated by the doctor.

I. After completion of the parent training and related forms, the study staff will undertake the venipuncture.

10.6.7 Venipuncture Procedure

For the required blood collections, the physician, after proper hand washing and restraint of the child, with assistance from study nurses, will locate the vein, clean the area with 70% isopropyl alcohol, allow it to dry, and collect the blood sample into sterile vacuum tube. Parents are free to remain present for the blood collection.

10.6.8 Vaccination Procedure

First, the injection nurses close the "injection cubicle" while the participant and parent remain outside. They open the **allocation group randomization envelope** (see section 10.4.4.3) for that child's dose (green for dose 1, yellow for dose 2, red for "insurance" dose 3). One injection nurse then shows the card to the other to confirm the dosage and method. Following the details described in section 10.4.2.3 above, the nurse duly prepares the syringe or jet injector cartridge, covers its tip to maintain cleanliness, and sets it down upon a clean surface and covers it.

The child is then brought into the "injection cubicle" and the parents are requested to remain outside. The **INF** vaccine is administered in the dose and route (ID or IM) specified by the randomization card into the anterolateral portion of the left thigh for children less than 12 months of age, or to the left deltoid muscle for children 12 months and older.

Immediately after the vaccination, the injection nurse will observe for several seconds the injection site for any wheal or bleb and any drops of blood or clear liquid, apply a band-aid, and duly note the information in the CRF, along with the time of injection. The ***allocation card*** is then destroyed and its pieces disposed into a paper shredder, and any remaining injection supplies or equipment removed from view.

The parent(s) and child are then reunited for holding and consoling (in the waiting area) during the subsequent minimum of 30 minutes of observation before reexamination of the injection site by the injection nurse (to avoid unblinding of the physican investigator from any temporary skin dimpling from the injector flange). During the post-vaccination waiting period, the parent/guardian will receive a final briefing and further training for aspects covered in section 10.6.4 above and reminded when to return next to the study center.

At least 30 minutes following vaccination, the injection nurse will note any immediate or early local adverse events and complete the remaining data in parts 7, 11, or 15 of Case Report Form (CRF) ("Medical Exam and [1st/2nd/3rd] Vaccination") and questions 1 through 8 of Parts 8 and 12 of the CRF ("Local Immediate Reactions"). In the event of an unexpected immediate local reaction – such as frank bleeding or laceration – the physician will be notified to institute needed medical care, take a photograph, and make appropriate entries in the adverse event section of the CRF. In all cases, the physician investigator will assess questions 10 through 15 of CRF parts 8 and 12 ("Immediate Systemic Reactions"). The parent and participant will then be discharged from the study center.

10.6.9 Rapid Diagnosis and Virus Culture for Influenza-Like Illness

It is useful to know the strains of Influenza A virus circulating in the community during the study, as well as to know which antibody responses in participants may be the result of infection with wild virus and not induced by the vaccine. Any participants who develop influenza-like illness (ILI) during the course of the study, either during their individual phase of active surveillance, as well as after they have "graduated" from the trial, shall have nasal and pharyngeal specimens collected for immediate rapid diagnosis of influenza A versus B. Simultaneous specimens placed in virus culture transport tubes will be quickly frozen and stored at -70°C. Frozen specimens from participants testing positive for influenza A will be shipped on dry ice to a laboratory for virus culture and typing.

The criteria for defining influenza-like illness that will prompt influenza rapid test and virus culture are listed in **Table 8**.

Table 8. Definition of "Influenza-Like Illness"

A. Temperature ≥38.3° C
And
B. 2 or more symptoms of
B1. Cough
B2. Sore throat
B3. Rhinitis or runny nose
B4. General malaise
And
C. No other obvious explanation for the fever
And
D. The symptoms have persisted 4 days or less

10.6.10 Specimen Preparation, Labeling, Storage, and Shipping

For use in the study, the "CDC and ATSDR Specimen Packaging, Inventory and Repository" (CASPIR) facility for receiving, processing, and shipping medical

specimens (http://www.cdc.gov/od/ads/caspir/), will prepare preprinted sets of peel-off, freezer safe, specimen labels meeting its guidelines. Each label will contain its own unique CASPIR-assigned serial no. and barcode.

10.6.10.1 Serum Specimens

For collection of serum for antibody assay, sets of 12 adjacent labels will also be labeled with identical barcoding and printing of the study designation code + study number + participant identification number, followed by the character "A" to link and identify blood collection tubes and aliquots from the first blood draw on day 0. Similarly, 12 labels for that participant will be printed with "B" to link and identify specimens from the second blood draw on nominal day 28, as well as another 12 labels printed with "C" for the third blood draw on nominal day 56.

Each label within each series of 12 (A, B, and C) will also be numbered and barcoded sequentially with "aliquot-01", "aliquot-02", and so on up to "aliquot-12". The highest aliquot number(s) will be affixed to the appropriate box on the CRF for that blood specimen, and onto other documents as needed.

The 5 mL specimens of whole blood to be collected on day 0, day 28, and day 56 for each participant will be prepared into serum the same day as drawn. The DEI/HIRRC laboratory will divide them into sequential cryovial tubes to be labeled starting with "aliquot-01", "aliquot-02", etc. Each tube will contain only 300 µL (0.3 mL) volume until the serum is used up or aliquot-08 is reached, which shall contain the remainder of serum available. If less than 600 µL total serum is available, it should be divided into only two aliquots. Serum should be stored at -70º C (with emergency power generator backup). Storage as warm as -18º C is acceptable in case of emergency.

At periodic intervals determined by the investigators, one half of the total aliquots from each participant for each draw will be consolidated into a subset for shipment on a Monday or Tuesday only, during non-holiday weeks, by overseas dry-ice air freight service to the CASPIR[g] for interim and long-term storage and eventual transshipment to the study assay laboratory (see section 10.6.12). Shipments from the Dominican Republic will be arranged and handled by experienced international medical specimen shipment companies, e.g., OCASA (universalpack@verizon.net.do), World Courier (http://worldcourier.com/us/indexsz.html) or Quick International (http://www.quickintl.com/).

While the first subset of aliquots is being shipped, the remainder shall remain at HIRRC in case of damage or loss in shipment of the first set. After the first set is received successfully at the specimen storage facility, the remaining aliquots will

[g] Shipment delivery address: CDC CASPIR, 602 Webb Gin House Road, Lawrenceville, Georgia 30045-5427 USA, Tel: +1 770-339-5950, Fax: +1 770-339-5943, Contact person: Robert J. Davidson, rum8@cdc.gov.

then be shipped. Similar procedures should be followed for phase II specimens, except interim shipments may be made at the discretion of the study investigators. Upon the direction of the U.S. Principal Investigator, CASPIR will transfer specified aliquot(s) to the designated laboratory for serologic assays.

Serum remaining from the study after testing shall be kept in long-term storage by the CASPIR specimen repository and by the testing laboratory. They may be transferred elsewhere upon request by the CDC or HIRRC investigators for additional testing or permanent storage, in accordance with the provisions of the informed consent. Such specimens shall remain the joint property of CDC and HIRRC, and should not be disposed or tested without their mutual consent.

10.6.10.2 Virus Culture Specimens

CASPIR will also prepare sets of 8 duplicate-numbered stickers for use in linking and identifying nasal and pharyngeal specimens collected into virus culture transport tubes and kits for rapid influenza testing. One label from each series will be inserted into the appropriate box on the CRF. These specimens will be collected only when participants develop influenza-like illness, in order to determine which strains of influenza A may be circulating during the study period.

10.6.11 Data Coding, Handling, and Analysis

As detailed in section 10.4.4 above, the blinding codes will be maintained in three secure locations by the independent party and other member(s) of the Data Safety Monitoring Board, as well as kept under seal by the HIRRC hospital director (in the BLUE envelopes, see 10.4.4.4). Codes will not be shared ("broken") with the study investigators (except as described in 10.4.4) until all clinical aspects of the study are completed and missing data obtained, and the serologic assays, performed blind, are completed and results shared.

Case Report Forms, Parent Diary, and other data recording sheets and forms from the study will be duplicated, with the originals kept in the HIRRC and complete copies furnished and maintained for analysis and preservation by the CDC. Data entry and analysis will be performed at CDC, with sharing of programming and data files with HIRRC (and with other parties at the discretion of the principal investigators once blinded laboratory assays are completed and results provided to CDC and HIRRC).

Once a CRF and its related forms are completed, verified, collected, and copied by the Dominican Principal Investigator, no additional information may be added to the originals. Any additions or corrections to the data of the CRF and related forms shall be made by means of the Data Clarification Form (DCF). This form (sections 11.4 and 13) shall be used to track inquiries to the clinical investigators from the sponsor (CDC) about entries in data fields of the Case Report Form, and/or to explain any subsequent changes in the data by the clinical investigators at HIRRC.

10.6.12 Laboratory Assays

10.6.12.1 Serology

Serologic assays for the study will be performed by a facility selected through WHO tender from among qualified research level institutions. For example, invitations will be extended to the Laboratory for Specialized Clinical Studies, Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA, as well as to the National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK.

Hemagglutination inhibition assays (HI) against viral strains similar to those included in the vaccine studied will be performed according to current Good Laboratory Practices (cGLP) and standard assay protocol. [Palmer1975, Lennette1995] Neutralization and/or other assays, may also be performed as valid indications arise and resources permit.

Subject to agreement for justifiable deviations, specimens will be handled and tested as follows: Upon arrival in the serology laboratory, sera will be stored at -20°C or colder until analyzed. Specimens will be treated at a dilution of 1:4 with receptor-destroying enzyme (RDE, *Vibrio cholerae* abnormal type 558 strain RDE; Denka Seiken Co., Ogdensburg, N.Y. and Tokyo, Japan, or equivalent) to eliminate nonspecific inhibitors, and then incubated for 16-18 hours in a 37°C H_2O bath. This will be followed by a second incubation for 30 minutes in H_2O bath at 56°C in order to inactivate the RDE.

After RDE inactivation, the HI process proceeds by diluting serum already at 1:4 in serial 2-fold steps (4, 8, 16, 32, 64, 128, 256, 512…) until a final dilution of 1:1024. Each specimen is separately diluted 1:4 and 1:8 for use as serum controls on each plate in which the specimen is tested. In addition to the serum controls, a red blood cell (RBC) control is also included on the plate for each specimen analyzed. At this point, 0.025 mL is transferred to duplicate rows of one test plate for each virus antigen to be analyzed. This yields four specimens per test plate with nine usable test dilutions, two serum controls and one RBC control in each of the duplicate rows.

The prepared, specified virus strain antigens are added to the nine test dilutions of every specimen and 0.025 ml of phosphate-buffered saline (PBS) is added to the serum and RBC controls. Each day assays are performed, the antigen is prepared to contain 4HA units/0.025ml. Virus strain antigens may be supplied from external reference laboratories, or prepared on site by the laboratory.

The antigen-serum mixtures are incubated at room temperature for 30 minutes. Next, 0.050 mL of a 0.5% chicken RBC solution is added to each well. Turkey RBCs may be used if chicken RBCs are not compatible with a specific virus antigen. Upon adding the RBC solution, the plates are incubated for 30 minutes at

room temperature, after which they are tilted and read. The titer is the reciprocal of the last dilution that shows complete inhibition of agglutination.

10.6.12.2 Rapid Test for Influenza Illness

The Microbiology Laboratory of the HIRRC will be supplied with rapid diagnostic tests capable of distinguishing influenza A from B (e.g., Remel Xpect® Flu A&B, Quidel QuickVue® Influenza A+B, Becton Dickinson Directigen™ Flu A+B, or equivalent). Nasopharyngeal and/or oropharyngeal specimens will be collected from participants with Influenza-like Illness (section 10.6.9) using swabs, collection kits, and instructions provided or indicated by the test involved. Participants who develop influenza-like illness multiple times during the study period may be tested multiple times as well. Specimens will be promptly assayed according to the manufacturer's instructions.

10.6.12.3 Influenza Virus Culture

The Microbiology Laboratory of the HIRRC will be supplied with virus culture collection and transport tubes with Hank's medium (e.g., BD Cellmatics™ Viral Transport Pack, Remel MicroTest™ M4®, or equivalent). These contain swabs with Dacron® tips and aluminum or plastic shafts to avoid damage to viruses present from calcium alginate or cotton tips and wooden shafts. Nasal and throat samples will be combined and stored at -70°C until it is determined by positive findings for influenza A to ship them to a specialist virology laboratory for culture and serotyping. The laboratory to perform such cultures will be determined by competitive tender by WHO among qualified reference laboratories.

10.6.13 Termination Visit

The final study visit will occur at ~ 56 days after dose 1 of **INF** vaccine, which should be approximately 28 days after dose 2. At this time, the 3rd blood draw and a 3rd ("insurance") dose of **INF** will be administered. If the participant does not return as scheduled for this visit, followup will be performed by home visit by study personnel.

10.7 Data Quality Assurance

10.7.1 Clinical Procedures

Personnel of CDC will visit the study site prior to initiation of the study to review with the site personnel information about the investigational agent, protocol requirements, randomization procedures, case report forms (CRFs), monitoring requirements, needle-free injection techniques, and reporting of serious adverse events.

10.7.2 Good Clinical Practices Monitoring

At visits during and after the study, the site will be monitored by CDC for compliance with current good clinical practices (cGCP) and other requirements, including accurate and complete recording of data on CRFs, source documents, and drug accountability records.

10.7.3 Data Handling

Case report form data will be entered in duplicate at CDC, using EPI-INFO (CDC, Atlanta, GA), Microsoft Access® (Redmond, WA), and/or equivalent software. Analysis will be performed by EPI-INFO, SAS™ (SAS Institute, Cary, NC), or equivalent software. Analyses will be performed by a predefined analysis plan.

10.8 Statistical Methods and Determination of Sample Size

10.8.1 Statistical and Analytical Plans

10.8.1.1 Primary Efficacy Analysis

The primary analysis of efficacy will be comparison of the proportion of participants within each investigational route of administration – ID-JI-0.1 and IM-NS-0.1 – achieving seroconversion one month after the second dose of vaccine to each vaccine strain versus the control needle-syringe full-dose route (IM-NS-0.25). As defined in section 10.5.1.2, the criteria for ***seroconversion*** to a vaccine strain will be a hemagglutination inhibition (HI) assay titer of 1:40 or greater for those with initial undetectable titers, or an increase of 4-fold or greater from a measurable initial titer to final titer.

Each reduced-dose investigational study arm, ID-JI-0.1 and IM-NS-0.1, will be considered an acceptable alternative method of vaccination if there is no more than a twelve percent decrement in the proportion of participants seroconverting compared to that of the full-dose, IM, needle-syringe control vaccination comparator (IM-NS-0.25) (*non-inferiority* model).[FDA1998, Chow2002]

10.8.1.2 Secondary Efficacy Analyses

Secondary efficacy analyses will compare the three study arms on the following:

a. proportion seroconverting one month after the first dose of vaccine, but before the second dose

b. proportions achieving seroprotection one month after both the first and second doses of vaccine. ***Seroprotection*** is defined as HI titers of ≥1:40, including among those pre-immune participants with detectable titers before vaccination.

c. geometric mean titers (GMTs) of HI assays performed one month after both doses 1 and 2.

d. satisfying the clinical criteria of the European guidelines for annual vaccine registration for adults aged 18 to 60 (there are none for children), [CPMP1997] that antibody responses to at least one strain of the vaccine achieve:

- >40% seroconversion among participants
- >2.5 increase in inverse geometric mean titer from pre-vaccination to final titers (e.g., from inverse GMT of 8 initially to final of ≥20)
- >70% seroprotection among participants

e. proportion with prompted and unsolicited adverse events following immunization.

10.8.2 Determination of Sample Size

The total participants of the combined phases I and II (N=450), results from 150 participants in each of three study arms. This number results from rounding up, in anticipation of an estimated 5% loss-to-followup, from a calculated 142 participants (*non-inferiority* model [FDA1998, Chow2002]) or 139 participants (*superiority* model) required per arm, derived using PASS 2002™ software (Number Cruncher Statistical Systems, Kaysville, UT, http://www.ncss.com), [Hintze2005] as follows:

The full-dose, standard IM route control arm of the study (IM-NS-0.25) is assumed to achieve the mean seroconversion rate against the A/H1N1 strain (86.4%) demonstrated in 1 published [Gonzales2000] (76%) and 3 unpublished [Saliou2005] recent clinical trials (~88%, ~79%, ~97%) of Vaxigrip® influenza vaccine (Sanofi-Pasteur, Lyon, France) conducted among a similar age range (6-35 months) of children as those to be studied in this trial (6-24 months). (Immunogenicity for at least one strain satisfies European Committee for Proprietary Medicinal Product requirements for European registration. [CPMP1997]) This rate is somewhat lower but consistent with the 91% seroconversion reported after use of a different influenza vaccine, Fluzone®, manufactured by the same company at its Swiftwater, PA, USA plant. [SanofiPasteur2004, Hoberman2003]

Based on prior literature indicating equivalent serologic responses between standard, full-dose, needle-syringe influenza vaccination and (1) reduced-dose, needle-syringe intradermal (ID) vaccination (see section 9.4.2), as well as (2) reduced-dose jet injected (JI) ID vaccination (see section 9.4.4), the same 86.4% seroconversion rate is postulated for the investigational intradermal study arm.

There is a paucity of and some conflicting results among clinical trials of reduced-dose influenza vaccination comparing the same antigen dosage by the ID versus IM or SC routes (see section 9.4.3). Dose-sparing trials without an ID arm showed a small decrement in response, [Treanor2002] so reduced dosage via the standard IM route is suggested as a useful strategy in time of shortage. [Treanor2004] Others have called for careful clinical trials to confirm the effect of the ID route. [LaMontagne2004] To fulfill this call

and clarify the issue, the reduced-dose IM arm is included in this trial with the same statistical assumptions as per the ID arm.

Applying a *non-inferiority* model using a score test for two independent proportions,[Farrington1990] a margin around these expected seroconversion rates (0.864) of twelve percent is selected, i.e., 0.104. This corresponds to an *acceptable* seroconversion rate as low as 0.760 (76.0%) in the investigational study arms, to demonstrate that the investigational arms are not worse than the control arm by more than this amount. (Decrements of up to 15% are suggested for non-inferiority trials in which the standard treatment rate is between 80% and 90%.[Chow2003]) Model parameters were set at:

target α = 0.05

Power = 0.80 (β = 0.2)
Standard group proportion (P2) = 0.864 (from prior studies)
A*ctual experimental group proportion* (P1.1) = 0.864 (anticipated)
Equivalent experimental group proportion (P1.0) = 0.760 (lowest acceptable)
Equivalent margin difference (D0) = -0.104 (acceptable twelve percent decrement)
Actual margin difference (D1) = -0.104 (acceptable twelve percent decrement)
Experimental group sample size (N1) = 142
Standard (control) group sample size (N2) = 142

The null hypothesis $\mathbf{H_o}$ is that the seroconversion rate for the treatment arm is *at least 12%* lower than for the standard arm, and that under the alternative hypothesis $\mathbf{H_a}$, the difference in seroconversion rates between the treatment and standard arms is *less than 12%* (i.e., the treatment is not inferior to standard). If the study rejects the null hypothesis $\mathbf{H_o}$ and accepts the alternative $\mathbf{H_a}$, we may conclude that the treatment is not inferior to standard, with only a 0.05 probability of reaching this conclusion due to random error.

Applying an alternative *superiority* model for two independent proportions (null case) using a one-sided Z test with pooled variance, [Chow2003] a sample size of 139 or greater in each group will detect with 80% power (β = 0.2) a superiority of ten percent or greater (seroconversion ≥95%) for either of the investigational study arms over the control arm (assumed 86.4%). As for the non-inferiority model, above, the significance level of the test was targeted at α = 0.05.

10.8.3 Randomization

As detailed in section 10.4.4, participants will be allocated to one of the three treatment groups (ID-JI-0.1, IM-NS-0.1, IM-NS-0.25) by a random allocation method not subject to observer choice or whim. Participants will be enrolled in blocks of six, each containing two members of each of the three treatment groups, in random order. One

block will be randomly split between the first and last enrollees in the study, to prevent block counting.

10.9 Stopping Rules to Suspend Vaccination

10.9.1 Phase I

During phase I (n=48), upon the occurrence of ***Serious Adverse Events*** in three (3 [6%]) or more enrolled vaccinees, or incidents of ***entire-limb swelling***[Rennels2000] or other ***severe local reactions*** at the **INF** injection site in five (5 [10%]) or more vaccinees, which, in the opinion of any of the Principal or Senior Dominican or U.S. Investigators are ***possibly-related*** or ***probably-related*** to the investigational vaccination (see section 10.5.2.2 for definitions).

Further **INF** vaccination for all participants will be suspended immediately until review of the data by the DSMB. Upon analysis using unblinded access to the study group code allocations, the DSMB shall provide either its clearance to resume vaccination, with restrictions, if any, as appropriate, or its denial to proceed. (The IRBs will be contemporaneously informed of any such suspension, as well as the outcome of the DSMB review, and the investigators will adhere to any resulting IRB guidance.)

10.9.2 Phase II

In accordance with the same criteria and procedures as described for Phase I in section 10.9.1 above, in phase II (n=402), all vaccination shall be suspended, upon the occurrence of ***possibly-related*** or ***probably-related Serious Adverse Events*** in sixteen (16 [4%]) or more vaccinees, or ***possibly-related*** or ***probably-related entire-limb swelling*** or other ***severe local reactions*** at the **INF** injection site in twenty (20 [5%]) or more vaccinees.

10.10 Criteria for Proceeding to Phase II

Upon unblinded analysis by the DSMB (see section 10.11) of the results of the phase I study, the observed adverse reactions will be tallied by the DSMB for each of the study arms (control IM-NS-0.25, investigational IM-NS-0.1 and ID-JI-0.1). Either or both investigational study arm(s) for which an observed numbers of reactions exceeds the number observed in the IM-NS-0.25 control arm by the following degree for any reactions listed will be eliminated from the phase II study. If both investigational arms are thus eliminated, the study will not proceed to phase II.

	Excess = no. in investigational arm less no. in control arm
Serious Adverse Reaction, *possibly*- or *probably-related*	≥3
Fever >40º C, *possibly*- or *probably-related*	≥4
Injection site whole-limb swelling	≥4

Injection site induration >5 cm in diameter	≥5
Injection site erythema >5 cm in diameter	≥5
Injection site pain level 3 (cries upon moving limb)	≥5

For example, if four (4) children in the IM-NS-0.25 control arm experience induration of >5 cm, eight (8) do so from the IM-NS-0.1 arm, and nine (9) from the ID-JI-0.1 arm, then the IM-NS-0.1 study arm may proceed to phase II, but the ID-JI-0.1 arm will be dropped from phase II.

Based upon this analysis and any other relevant information, the DSMB will decide whether or not the study should proceed to phase II as designed, should be modified and then proceed, or should be terminated. This decision shall be duly informed to the investigators, without revealing unblinded information nor study-group-specific analyses. Regardless of a DSMB decision permitting phase II, the study sponsor may elect for any reason not to proceed, and to terminate the study after phase I.

10.11 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be appointed in advance of the study (see section 8.8). Its members will certify no financial or related interests in any of the organizations conducting or cooperating with the study: CDC, HIRRC, WHO, PATH, or Bioject. Any disclosed exceptions to the certification shall be considered by the DSMB prior to material discussions so that they may be reflected in the minutes along with the DSMB's determination how to handle such exceptions. The group expertise will include the fields of vaccine science, immunization, pediatrics, public health, statistics, and clinical medical research. Statistical expertise will be provided by Maria Deloria Knoll, PhD, DSMB Chair, who will be assisted, as needed, by fellow faculty and programmers of the Johns Hopkins University School of Public Health.

In advance of implementation of the study, members of the DSMB will each be provided with drafts of key study documents (protocol, consent form, case report form, diary forms, etc.) for an opportunity to review them and suggest changes on the basis of their scientific expertise and personal experience. One or more of the DSMB members will assist in preparing the allocation cards and envelopes used to randomize consecutive participant ID numbers into the three study arms. During the blinded period of the trial the DSMB will maintain in at least three separate locations the coding list to identify these allocations by study ID number.

The DSMB shall meet formally by telephone conference call at least four times: (1) prior to initiation of the study, (2) during the phase I enrollment period, (3) upon being provided with blinded clinical data from the completion of the phase I clinical aspects, and (3) upon being provided with unblinded analysis following the completion of clinical and laboratory analysis from phase II. In addition, the DSMB may meet for any requested *ad hoc* meetings as described above, or upon its own initiative for cause.

Upon the incidence of SAEs which trigger the criteria for suspending vaccination, as per section 10.9, the DSMB will analyze the situation in unblinded fashion using the coding sheets, and make its recommendation to the investigators whether or not vaccination may resume or whether the study -- or one of its investigational study arms -- should be terminated.

At the conclusion of phase I, the DSMB will be provided by the investigators with adverse events and other results from the phase, linked to study ID number. As per section 10.10, the DSMB will link this data with the study allocation group information from the coding sheets and analyze them for purposes of applying the rules for proceeding to phase II, and so inform the investigators of their conclusions.

Upon the completion of the blinded clinical and serologic aspects of the study, after either a termination following phase I or upon completion of phase II, upon request the DSMB will furnish the investigators the coding lists so that unblinded analysis of the results may occur.

10.12 Early Reporting to DSMB and IRBs

The conjunction of certain adverse events (AEs) and unanticipated problems will be reported promptly as they occur to the DSMB and both IRBs (see definitions for terms in section 10.5.2.2) These shall include:

- Any *serious* AE (SAE) that is also an *unanticipated* AE, and is *probably-related* or *possibly-related* to participation in the research.
- A series of *serious* AEs that are expected in some participants, but in the judgment of the investigators are occurring at a significantly higher frequency or severity than expected.
- Certain *unanticipated* AEs, regardless of severity, that might alter the IRB's analysis of the risk versus potential benefit of the research *and*, as a result, might warrant substantive changes in the research protocol such as the informed consent process and form, exclusion criteria, monitoring methods, or termination of enrollment.
- *Unanticipated problems that do not involve adverse events*, but which may have increased the risk of past or future harm to participants

In the event of any such occurrences, the Dominican investigators will document the situation in writing and notify promptly by telephone, fax, or email both the FDI IRB and the U.S. investigators, who shall notify the CDC IRB and the DSMB members, and at their discretion, the vaccine and jet injector manufacturers.

10.13 Changes in the Conduct of the Study or Planned Analyses

Only the sponsor (CDC, represented by the U.S. Principal and Senior Investigators) may modify the protocol. Amendments to the protocol will be made only upon consultation and agreement between the sponsor and the Department of Infectious Diseases of the HIRRC (represented by the Dominican Principal and Senior Investigators), with consultation from

the other cooperating institutions. The only exception is when a clinical investigator considers that a participant's safety is compromised without immediate action. In such circumstances, immediate approval of the chairman of the FDI Institutional Review Board (IRB) or ethical committee (EC) must be sought, and the investigator should inform the sponsor and the full IRBs or ECs within 5 working days after the emergency occurred.

All amendments that have an impact on participant risk or the study objectives, or require revision of the informed consent document, must be notified to the DSMB (see section 10.11) and receive approval from both the CDC and FDI IRBs or ECs prior to their implementation. The IRBs or ECs will also be routinely notified of any minor or administrative changes in the protocol. The DSMB shall also be informed and advised on major study modifications affecting scientific integrity or participant safety, such as increasing sample size.

11. Policies and Practices

11.1 Compensation, Insurance, and Indemnity

Study participant parents/guardians will receive monetary reimbursement to cover estimated transportation costs from home to the hospital for the initial recruitment trip and all follow-up visits related to the study. They will also receive a voucher for each visit entitling them to a snack lunch at the hospital snack bar to compensate for the extra time away from home required by the study. They may also receive a waterproof folder or carrying case for storing the participant diary, the thermometers, the circumference tape, the local reaction measurement template, and any other study materials provided to them.

The study participants will be entitled to necessary outpatient and inpatient medical care at the HIRRC similar to that available to other patients at this public institution, and at no charge for any adverse results or consequences of their participation in the study (see section 7.4).

In addition, an insurance policy will be purchased after all approvals are received from the IRB and other necessary parties to cover liability of HIRRC, CDC, and WHO for harm or damages resulting to participants from involvement in the study. The coverage will be purchased by the WHO from a reputable international insurer of clinical research worldwide, such as Biomedic-Insure (http://www.biomedic-insure.com/indexus.htm, Vannes, France), or equivalent company. Coverage shall be approximately €800,000 per protocol and a sublimit of about €160,000 per participant, equivalent as of 23 February 2006 to US$953,754 (RD pesos 32.8m) per protocol and US$190,737 (RD pesos 6.57m) per participant.

A summary of the above compensation, entitlement, and insurance will be mentioned in the informed consent form, but the policy amounts will NOT be disclosed, and the participant's parents will specifically be informed that coverage is for liability of the insureds to the participants, rather than direct coverage for health claims of the participants.

11.2 Study Material Accountability

The study investigators at the HIRRC must maintain accurate records of dates and quantities of product(s) received, to whom dispensed (subject-by-subject accounting), and accounts of any product accidentally or deliberately destroyed. The investigator must retain all unused or expired study supplies until confirmation of the accountability data by the sponsor.

11.3 Case Report Forms

Case report forms will be provided for each participant. Correction to data on CRFs may be made only by putting a line through the incorrect data and writing the correct values, allowing the original text to remain legible. Each correction must be initialed and dated by the person making the change. If corrections are made after review and signature by the HIRRC investigator, he or she must be made aware of the changes and document this awareness.

It is expected that the CRF will serve as the official medical record into which most data about the participant for this study is primarily or exclusively entered. It is the policy of this study that any other secondary data which is copied from other records into the CRF must be verifiable to the source data. This necessitates access by the sponsor, CDC, to all original recordings, laboratory reports, and participant's records. The HIRRC investigators agree to allow access to participant records and other sources for all study data, which will be maintained in confidence by CDC. The participants (or their legal representatives) must also allow access to the participant's medical records, and they will be informed of this and will be signing their agreement when giving informed consent.

11.4 Data Clarification Forms

Investigators and collaborators may have questions about the Case Report Form data after copies of the CRF data are transmitted from the study site to the sponsor. These questions will be submitted and answered by documenting them with **Data Clarification Forms** (DCFs). There are two types of DCF inquiries, *internal* and *external*:

Internal DCF: A query involving matters OTHER than safety, adverse events, and primary endpoint data, for which the resolution is self-evident. It can be answered without changing the meaning of the data (such as moving data incorrectly entered into one box on the form into another, correct box). It can be answered using logical numeric flow (for example, if the serial reporting number of an adverse event is missing, the next number in sequence can be assigned). Any of the U.S. (Sponsor) or Dominican investigators can create and answer an internal query. The U.S. Investigators will send the Dominican investigators a copy of each internal DCF completed by the Sponsor. The Dominican investigators will do the same for the U.S. Investigators on internal DCFs completed by the Dominican Investigators. The recipients will review the internal DCFs received, and if he/she disagrees, he/she will notify the other party, who will then generate a new DCF documenting the investigator's correction.

External DCF: A query from the U.S. Investigator (Sponsor) that requires information from the Dominican Principal Investigator, particularly on key subjects such as safety (e.g. the relationship of an adverse event to vaccination) or data on a primary endpoint (immune response, specimen identity). The Dominican investigators will provide the answer on an external DCF, sign it, and return it to the Sponsor.

11.5 Study Monitoring

In satisfaction of common regulatory requirements and *current good clinical practice* (cGCP), all aspects of the study will be monitored by designated investigators of the sponsor, CDC. For such monitoring purposes, the HIRRC investigators agree to allow access to the clinical supplies, dispensing, storage areas, and the clinical files of the study participants, and, if requested, agree to assist in such monitoring.

In certain circumstances, a secondary audit may be conducted by the sponsor, CDC, or its designee. The HIRRC investigators will be informed if this is to take place and advised as to the nature of the audit.

11.6 Retention of Records

As required by applicable regulations, HIRRC investigators must retain all study records in a secure and safe facility. The HIRRC investigators must consult a representative of the sponsor, CDC, before intended disposal of any study records, and must notify the CDC of any change in the location, disposition, or custody of the study files. The period of record retention should be consistent with the record retention policies of the Dominican Republic, or the Committee for Proprietary Medicinal Products (CPMP) of the European Agency for the Evaluation of Medicinal Products, whichever is longer. The CPMP requires retention for the maximal period of time permitted by the institution, but not less than 15 years. HIRRC shall destroy the Participant Identification Form containing confidential information at the end of any applicable retention periods, or after 15 years, whichever is later. CDC shall retain for 15 years copies of those anonymous records furnished to it by HIRRC for data entry and analysis purposes.

11.7 Use of Information and Publication

The principal parties to and collaborators with the study -- HIRRC, CDC, WHO, PATH, Bioject, and the serology laboratory -- recognize the importance of communicating medical research results and therefore each encourages publication of the results of this study in reputable scientific journals and at seminars or conferences.

All parties agree to furnish the others with a copy of any proposed public presentations or publication related to the study. The comments thereon shall be replied to the submitting party without undue delay, and not later than within 60 days. If comments or suggestions by any of the parties are not accepted, the senior author of the abstract or manuscript and representatives of the other parties shall promptly meet and endeavor to agree mutually on the final wording and disposition of the publication. In the event such agreement is not

achieved, the author(s) of any abstract, presentation, or publication shall duly disclose therein the objections of the other party(ies). No participant in this research may be listed as an author or co-author on a publication or conference abstract presentation without their prior consent. The same procedure and policy shall apply to publication or presentation of the results of prematurely discontinued and any other noncompleted aspects of this study.

Due regard shall be given to the legitimate interests of Bioject and any other manufacturer whose products are used in the study. Such interests include technical accuracy, interpretive conclusions, manuscript co-authorship, obtaining patent protection, coordinating and maintaining the proprietary nature of submissions to health authorities, coordinating with other ongoing studies in the same field, and protecting confidential data furnished in furtherance of the study.

Unpublished results from investigations shall not be made available to any outside party not otherwise involved in the study by the investigating team without similar use of the presentations and publications procedure as outlined previously. Neither Bioject nor any other manufacturer whose products are used in the study shall quote from publications by investigators in its scientific information or promotional material without full acknowledgment of the source (i.e., author and reference).

It is intended and understood by the Dominican investigators at HIRRC and the U.S. investigators at CDC that the information generated in this study may be used by the manufacturer(s) of product(s) used therein in connection with the development of its product and therefore may be disclosed to government agencies in various countries. To allow for the use of information derived from the study, within three months after the conclusion of the study and its data analyses, upon request, the HIRRC and CDC investigators will provide such manufacturer(s) with copies or summaries of clinical data and records, except confidential Participant Identification Form information.

No manufacturer furnishing products used in this study may make any claims to the public, in writing or in oral presentations, nor may it cite in its advertising nor product labeling, any statement suggesting or implying that HIRRC, CDC, WHO, or PATH in any way endorse the products. However, a manufacturer may state that it collaborated with these organizations in experimental use of its products. It may cite published results of the study. It may use unpublished results for regulatory or commercial purposes other than publication or advertising.

Specimens of serum, extracts, or isolates provided to laboratories under this protocol shall not be transferred to other parties without an express written *Materials Transfer Agreement* executed by HIRRC and CDC.

11.8 Statement of Obligations

11.8.1 Sponsor and Monitor

The sponsor (CDC) has already, or will:

A. Conduct one or more prestudy visits to:
   1. Establish the acceptability of the facility and record this in a written report (memorandum or form).
   2. Discuss the proposed trial with the clinical investigators at the HIRRC, supply the case report form, draft protocol, and other documents for review and approval.
   3. Discuss with the investigator regulatory requirements with respect to informed consent, IRB or EC approval of the trial, the protocol, including protocol amendments, and informed consent changes.

B. Conduct periodic site visits to:
   1. Assure adherence to the protocol.
   2. Review case report forms and medical records for accuracy and completeness of information.
   3. Examine pharmacy records for documentation of quantity and date of receipt of investigational supplies, dispensation and accountability data for administration to each Participant, loss of materials, contamination, and unused supplies.
   4. Record and report (summarize) observations on the progress of the trial and continued acceptability of the facilities in a site visit report.
   5. Review investigator files for required documents, e.g., protocols, protocol amendments, IRB or EC approvals (protocols, amendments, informed consent, etc), IRB charter and membership, and communications to and from the IRB or EC and the investigator.

11.8.2 Clinical Investigators

A. **Institutional Review Board or Ethics Committee**. The Dominican Principal and Senior Investigators must assure the sponsor or monitor that the IRB or EC of the Fundación Dominicana de Infectología (FDI):

   1. Meets regulations as defined in 21 CFR Part 56 of the U.S. Code and other applicable requirements.
   2. Has authority delegated by the parent institution, which authority is found in the by-laws of the IRB or EC, its operating guidelines or its charter, to approve or disapprove clinical trials and protocols, including informed consent forms and other documents (protocol amendments, information to be supplied to participants concerning informed consent, etc).
   3. Complies with proper personnel makeup of an IRB or EC.
   4. Convenes meetings using acceptable rules of order for making decisions, recording such decisions and implementing them.
   5. Files contain (a) documentation of its decisions such as are found in IRB or EC minutes and correspondence, (b) written guidelines or bylaws governing IRB or EC functions, (c) protocols, (d) protocol information

to be supplied to the subject, (f) correspondence between the IRB or EC and the investigator (consent changes, protocol amendments, etc).

B. **Informed Consent of Human Subjects**. The Dominican Principal and Senior Investigators must assure the sponsor or monitor that the informed consent form for participant subjects:

1. Meets regulations as defined in 21 CFR Part 50 of U.S. Code for Informed Consent, and other applicable requirements.
2. Has been approved by the FDI IRB or EC, including, when required, information to be given to the participant regarding the trial he or she is enrolled in.
    a. Informed consent includes the basic elements and any additional elements necessary.
    b. The participant and a study site representative sign the form and the participant is given a copy.

C. **Storage and Dispensing of Study Supplies**. The Dominican Principal and Senior Investigators (or pharmacist) must demonstrate to the sponsor or monitor that:

1. Adequate and accurate written records show receipt and disposition of all study supplies, including dates, serial or lot numbers, quantities received, and each quantity dispensed, administered, or used, with identification of each participant.
2. Purpose and reasons are given in written records for study material disposal, e.g., the amount contaminated, broken, or lost, and the quantity returned to the sponsor.

D. **Case Report Forms**. The Dominican Principal and Senior Investigators must assure the sponsor that:

1. Case Report Forms, when completed, accurately reflect the medical records on each participant.
2. Case report forms and medical records will be accessible to the sponsor or other authorized inspectors during site visits.

E. **Files and Records**. (See section 11.6.) The Dominican Principal and Senior Investigators must assure the quality, integrity, and content of his or her files that will be inspected by the sponsor, and any other authorized inspectors. The files must contain, as a minimum:

1. Correspondence to and from the IRB or EC and the investigator.
2. Documents that include:
    a. IRB/EC-approved protocols.
    b. IRB/EC-approved protocol amendments.

c. IRB/EC-approved informed consent and information supplied to the participant.
d. IRB/EC charter, membership, and qualifications.

3. Clinical supplies:
   a. Record of receipt, date and quantity, batch or lot number.
   b. Disposition dates and quantity administered to each participant.
   c. Inventory records.

12. Questions and Answers on Study Plan, Policies, Ethics

12.1 Why is this study being done in infants from 6 to 24 months of age, rather than first in adults or older children?

There is already a substantial literature documenting the clinical immune response and safety for the intradermal administration of inactivated influenza vaccine to adults (see sections 9.4.2 to 9.4.4), and in some studies to older children[Beasley1960,] or to children of unspecified age[Bruyn1947, Sigel1957] . But there is little[Bruyn1949b, Klein1961] or no data on intradermal influenza vaccination in the age group of infants 6 months to 2 years who are particularly susceptible to serious morbidity and mortality from this disease, and who are now recommended for routine universal vaccination in some developed countries.

The investigational spacer to be studied in this trial has already been subjected to a number of well-conducted studies on adults without serious adverse consequences (see section 10.4.3.6). Thus, it is reasonable now to study young children in order to gather essential data for policymaking on how such participants may be protected in the event of pandemic. This study is phased with conservative stopping rules that can quickly respond to any clinical outcomes suggesting untoward risks to these children.

12.2 Why is this study being done in the Dominican Republic and not in the United States or Europe, where its sponsor and principal parties are based?

This kind of study could and should very well be conducted in the U.S. or Europe, as the questions it poses are relevant to those geographic areas as well. However, the collaboration supporting this study benefits from the technical scientific and/or financial contributions of WHO and PATH, organizations with primary missions to seek answers to the health problems of the developing world. This global perspective is also within the mission of CDC, which recognizes that national health and security depends on the health and security of all nations. The selection of the HIRRC as a partner in this study resulted from its excellent track record of clinical trials of vaccines and antimicrobials (see section 10.2.1).

12.3 Why is the study planning to use Vaxigrip® instead of an influenza vaccine licensed in the U.S.A.?

The brand of influenza vaccine to use in this study was selected by a process of elimination from among the seven major products on the international market, of which three are now sold in the U.S.

One priority was to minimize the number of "investigational" aspects of the study by using an influenza vaccine already licensed and marketed in the Dominican Republic. There are three: (1) **Vaxigrip** (in prefilled syringes), (2) **Fluarix**® (GSK, manufactured in Dresden, Germany), and (3) **Agrippal® S1** (Chiron, manufactured in Siena, Italy). Well after planning for the study began, **Fluarix** was licensed in the U.S., but worldwide it is only available in prefilled glass syringes of 0.5 mL. Thus, using **Fluarix** would be wasteful for administering the 0.25 mL and 0.1 mL doses for the two needle-syringe study arms. Also, it would be impractical to transfer 0.1 mL from the glass **Fluarix** syringe into the male tip of the Biojector cartridge, requiring an expensive, disposable Luer adapter (and would still waste 80 percent of the vaccine left in the syringe). **Agrippal S1** is not licensed in the U.S., and its prefilled syringes would produce the same obstacles as **Fluarix**. **Agrippal S1** also comes in single-dose vials without preservative, which means they cannot be used to fill multiple syringes.

Neither (4) **Fluzone**® (Sanofi-Pasteur, manufactured in Swiftwater, PA), (5) **Fluvirin**® (Chiron, manufactured in Liverpool, UK), (6) **Begrivac™** (Chiron, manufactured in Marburg, Germany), nor (7) **Fluad**® (Chiron, manufactured in Siena, Italy) are licensed for use in the Dominican Republic. Moreover, **Fluvirin** is not licensed for use in ages under 4 years. **Begrivac** and **Fluad** are also adjuvanted products, which may increase reactogenicity when administered into the skin.

12.4 Why is the study not being carried out under Investigational New Drug (IND) application to the FDA/CBER because of the non-labeled ID route and reduced dosage?

The original intention for this study was to seek such regulatory oversight by FDA, as an indicator of the most rigorous standards of research quality, even though it is not technically required for studies outside the U.S. for vaccines not anticipated to seek U.S. licensure. However, consultations with Loris D McVittie (mcvittie@cber.fda.gov) of the Center for Biologics Evaluation and Research of FDA indicated that the agency would be unable to accept such a filing. This is because FDA has no documentation on file for the *chemistry, manufacturing, and controls* ("CMC") of Vaxigrip. Such information is very extensive and expensive to assemble, and is proprietary to the manufacturer. In the absence of any plans to bring this product into the U.S. market, Sanofi-Pasteur would not do so for this study.

The planned importation of multidose vials of Vaxigrip into the Dominican Republic for investigational use in this study will require the authorization of the national pharmaceutical regulatory authority of the Dominican Republic, the Dirección General de Drogas y Farmacias of the SESPAS [Ministry of Health]. In addition, such studies in its facilities must be approved by appropriate officials. Thus, SESPAS has both regulatory oversight and political authority over this study, and will be exercising it accordingly.

Before implementation, this study will undergo and require consideration and approval not only from the IRBs of CDC and the FDI, but also from appropriate review bodies of the Dominican Republic Ministry of Health (SESPAS), the World Health Organization (WHO), and the Program for Appropriate Technology in Health (PATH). Such reviews will inevitably apply rigorous ethical, scientific, and policy scrutiny that will more than compensate for the absence of FDA oversight via the IND process.

12.5 How will one know the amount of vaccine actually delivered into the skin by the intradermal spacer, versus going into deeper tissues, or dripping out onto the skin?

It is very difficult for any vaccination study to document the amount of vaccine that actually enters the intended target tissue, whether skin (ID), muscle (IM), or fat (SC). Indeed, there is evidence that a substantial proportion of intended IM injections likely end up in the fat. Health workers have been found to underestimate the fat pad thickness in selecting needle length, or do not insert enough of it.[Cockshott1982, Poland1997]

Proving the site of deposition or measuring the quantity of antigen reaching various tissue compartments would require contrast-imaging, biopsy, and similar expensive and invasive procedures. Regulatory agencies consider these unnecessary in most routine clinical trials because noninvasive measures and outcomes are available to document that the vaccine achieves its purpose. For ID injections, the presence of a wheal immediately after injection, and the absence of clear liquid drops on the skin, suggest that the dose went neither too deep nor too shallow. Both these variables will be collected in this study by observation immediately after vaccine doses 1 and 2 and noted on the Case Report Form.

In general for almost all vaccine trials, the immune response is empirical evidence of success. If antigen is placed into the wrong compartment in some participants, or partly escapes through the injection site, this would be an academic issue if participants are otherwise adequately protected from disease. If not, the immediate observations of the injection site may provide clues to any failure to effectively immunize, and provide a basis for further studies to elucidate the problem, if warranted.

12.6 Will the local side effects of intradermal injection be more painful for participants than needle-syringe?

The first studies of the Biojector with intradermal spacer were conducted by anesthesiologist professor Elemer Zsigmond at the University of Illinois (see 0), and suggested that ID injection was less painful than using a needle to administer lidocaine.[Zsigmond1999a, Zsigmond1999b] Other clinical studies have demonstrated favorable safety and tolerability.

Subjects in a Navy vaccine trial who received Biojector ID injections in one arm and Biojector IM in the other (see 10.4.3.6.2), reported overwhelming preference (26 vs. 3) for either Biojector route over their prior experience with needle-syringe (but no control needle injections were tested).

In a study of infants like this one, it is difficult to assess pain. However, parents and staff will be assessing local pain daily on the diary form and followup visit, by the participant's reaction when the limb is touched or moved.

12.7 Why is there no study arm of children receiving the reduced vaccine dose intradermally using needle-syringe?

Although it would be of intellectual interest to compared two different methods of intradermal delivery -- needle-syringe (N-S) and jet injector (JI) -- such a comparison was precluded for two reasons.

First, adding another study arm would increase the cost of the trial by about one-third, and would extend its duration by that same degree. Resources are insufficient to do so, and delaying the results is deemed inadvisable because of its potential importance for pandemic response. Second, the Mantoux method of needle-syringe injection into the skin is considered impractical for widespread use for reasons of speed, logistics, occupational injuries, and waste disposal burdens (see section 9.1). Thus the data would not be actionable.

12.8 Why was the Biojector system picked for use in this study, rather than other simpler, more economical jet injectors?

It is quite true that there are other needle-free jet injectors on the market or in development that are less complex in their construction than the Biojector, cost less to purchase and to buy their disposable cartridges, do not require CO_2 canisters which can be hard to procure in developing countries, and other reasons that might make them more suitable for use in developing countries than the Biojector.

Despite these disadvantages, however, the Biojector is the only one among them for which substantial data has been accumulated in bench, animal, and clinical

trials for the investigational intradermal spacer (see section 10.4.3). To try to study intradermal delivery of any of these other jet injectors would require substantial additional resources and delay in conducting such prior studies in order ethically to justify the type of study intended here.

This study can be considered a *proof-of-principle* trial. If needle-free jet injection is demonstrated a safe and effective means of dose-sparing for influenza vaccination, it would serve to encourage other jet injector developers and manufacturers to pursue this route. The general concept of creating a gap between the orifice and the skin at the nozzle of a jet injector in order to weaken its stream to achieve intradermal injection is an old one (see section 9.6.3) and no longer under patent protection. Thus, other manufacturers would have freedom to adapt their own devices with gaps of appropriate length. This would help to produce a competitive marketplace for safer, simpler, and swifter intradermal delivery by jet injection.

12.9 What is the justification for giving the third "insurance" dose earlier than the routine annual revaccination? What are the risks, particularly for the control group which has already received the recommended two full doses?

It would be ideal if we could know immediately the results of the final blood draw on day 56 (8 weeks), so that we might avoid the inconvenience and expense of a third dose that day in those children who already have protective levels of antibody against influenza. But that is not possible.

Another option considered, but not chosen, would be to break the code of random allocation at the time of the final blood draw and vaccinate only those who had received the investigational low-dose regimens by ID or IM. These two groups might be at higher risk of not being protected than the control group which received the standard regimen for age and would not be indicated for revaccination until the next season. But it was decided that breaking the code so soon would severely compromise the study design. It may bias ascertainment for any delayed adverse events that may occur in the weeks and months after the active surveillance phase ends on day 56.

The primary reason for vaccinating on day 56 is to ensure that all participants are protected promptly against influenza, to which they might be exposed in the months ahead while waiting for antibody levels are being determined.

Extravaccination is the term used for the administration of vaccine antigens, earlier than otherwise indicated, for which a patient may already be immune as a consequence of prior immunizations or natural immunity. The Advisory Committee on Immunization Practices (ACIP) of CDC, the American Academy of Pediatrics (AAP), and the American Academy of Family Physicians (AAFP) have made recommendations on this subject in their joint statement on combination vaccines (nominal pages 5-6, .pdf file pages 19-20 of 30: http://www.cdc.gov/mmwr/PDF/rr/rr4805.pdf). CDC1999, AAP1999, AAFP1999

To avoid missed opportunities to vaccinate in the era of newer combination vaccine, it sometimes becomes necessary to give additional doses of some antigens because they were combined with others indicated for the child at the time, and monovalent products may not be available. The statement recommended that giving non-indicated antigens might be justified when "potential benefits to the child outweigh the risk of adverse events associated with the extra antigen(s)".

ACIP/CDC, AAP, and AAFP pointed out that "an extra dose of many live virus vaccines and Hib or HepB vaccines has not been found to be harmful. However, the risk of adverse reactions might increase when extra doses are administered earlier than the recommended interval for certain vaccines (e.g., tetanus toxoid vaccines and pneumococcal polysaccharide vaccine)." They went on to write that:

> **"Inactivated Vaccines**
>
> "When inactivated (killed) or subunit vaccines (which are often adsorbed to aluminum-salt adjuvants) are administered, the reactogenicity of the vaccine must be considered in balancing the benefits and risks of extra doses. Because clinical experience suggests low reactogenicity, an extra dose of Hib or HepB vaccine may be administered as part of a combination vaccine to complete a vaccination series for another component of the combination. Administration of extra doses of tetanus toxoid-containing vaccines earlier than the recommended intervals can increase the risk of hypersensitivity reactions." CDC1999

The statement did not comment on extravaccination with influenza vaccine. The investigators for the present study are unaware of any clinical data on giving a third dose of influenza vaccine one month after completion of the recommended two-dose primary series. Unlike tetanus-toxoid and hepatitis B vaccines, however, the inactivated Vaxigrip influenza vaccine does not contain any irritating adjuvants. It is also "split" to reduce reactogenicity compared to a whole-virus vaccine. Annual influenza vaccinations are now routinely recommended for all infants in the U.S. in the age group to be studied here. This means that some infants and young children completing their primary series *at the end of the influenza immunization program period* might be revaccinated with their next, third dose as early as 6 months later, *at the beginning* of the next period, which would be in full compliance with ACIP/CDC recommendations.

Given this context, the researchers believe that the benefits of ensuring prompt protection from influenza for all the study participants and avoiding loss-to-followup by accelerating the next permissible third dose from 6 months later to 1 month later outweighs the anticipated routine side effects of the "insurance" dose.

12.10 Why is the final blood draw (day 56) conducted at the same time as the 3rd "insurance" dose of vaccine is received by the reduced-dose participants, rather than later? Why is serology not performed after receipt of this 3rd dose?

The 3rd dose of vaccine provided on day 56 to participants in the reduced-dose study arms of the trial is not provided to answer any research question of the study. Instead it brings the total dose of vaccine administered to the IM-NS-0.1 and ID-JI-0.1 study groups to 0.45 mL, essentially equivalent to the standard 0.5 mL administered over two doses to the control IM-ND-0.25 group. (It may also be more immunogenic than the standard regimen, as it was delivered in 3 doses over a longer interval.) Giving the 3rd "insurance" dose is a safer, quicker, more reliable, and less discomfiting means to ensure that the IM-NS-0.1 and ID-JI-0.1 children are protected against influenza, which is the major benefit for his or her participation in the study.

The alternative to the insurance dose would be to wait several months, or longer, for the serologic assays results to come in. These tests will be batched and run at the end of each phase to minimize dissimilarities in assay conditions. In such a scenario -- not pursued here -- those with inadequate immune responses would need to be called back for another dose. In the meantime, while waiting for serologic results, or waiting for notified parents to bring children back for revaccination, some of the reduced-dose children, having received only 0.2 mL of total vaccine (without the insurance dose), might have been unprotected all along, exposed to influenza, and become sick unnecessarily.

A key scientific question of the study is whether two intradermal doses at 0 and 28 days will protect the child. Thus the day 56 blood draw is a necessity, regardless of whether further vaccine doses ("insurance" and "bonus") are given for ethical reasons. Since three or more doses in a primary influenza vaccination series would not be a practical public health strategy for the general population -- as two pediatric doses are the accepted standard -- there is little or no scientific justification to document the antibody responses after two investigational doses plus the 3rd insurance dose.

Drawing blood from a young child's vein for diagnostic purposes is a discomfiting procedure for patient, parent, and physician alike. Although the results of a 4th blood draw in this study after the 3rd "insurance" dose might satisfy intellectual curiosity, without any valid scientific reason it would be unethical to subject the children to it. It would again, take some months for serologic assays to be performed and to call back any patients unprotected even after the insurance dose. Giving another dose of vaccine is less painful, less costly, and less logistically complicated than phlebotomy and overseas laboratory assays.

No routine vaccinations, particularly influenza, provide 100% seroconversion rates in all ages. Except in rare circumstances, such as proving rabies immunity

among veterinarians, standard clinical practice avoids the pain and societal expense of serologic assays to prove a vaccine has worked.

This is the reason for our providng a 4th "bonus" dose, 6 months after the final blood draw, on ethical grounds to provide protection against the next season's influenza for all children, including those whose response to the prior doses may have been suboptimal. For "annual" recommended revaccinations, six months is the standard minimum interval for a patient vaccinated at the tail end of one Northern Hemisphere season (March 30) and at the beginning of the next (October 1).

13. List of Associated Documents

- Protocol – Spanish version
- Informed Consent Form - Spanish
- Informed Consent Form - Additional Information - Spanish
- Informed Consent Form - English translation
- Informed Consent Form - Additional Information - English translation
- Participant Identification Form (PIF)
- Enrollment Log / Participant Inscription Form
- Study Staff Responsibilities and Signatures Registry and Log
- Case Report Form (CRF)
- Parent's Diary Form, Vaccination 1, Days 0 – 7
- Parent's Diary Form, Vaccination 1, Days 7 – 28
- Parent's Diary Form, Vaccination 2, Days 0 – 7
- Parent's Diary Form, Vaccination 2, Days 7 – 28
- Serious Adverse Event Form (SAE)
- Data Clarification Form
- Appreciation Certificate and Bonus Vaccination Reminder Form

14. References

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